Infection

ichroma™ ROTA

INTENDED USE

ichroma™ ROTA is a fluorescence Immunoassay (FIA) for the qualitative determination of Rotavirus in human feces. It is useful as an aid in screening of Rotavirus infection.

For *in vitro* diagnostic use only.

INTRODUCTION

Rotaviruses (RVs) are the main etiologic agents of serious diarrheal disease in infants and young children under 2 years of age throughout the world. For developing countries, approximately 125 million cases of RV infection occur annually in children under 5 years of age, of which 18 million are moderately severe to severe; almost 900,000 children die annually from RV infections in these countries.

For the USA it is estimated that RV infections cause an estimated 1 million cases of severe diarrhea and approximately 150 deaths per annually. [1)]

RVs are transmitted mostly by the fecal–oral route. A high degree of resistance to physical inactivation, the large number of virus particles shed, and the very low diarrhea dose 50% ensure that infection is also easily taken up from environmental sources, as demonstrated by tenacious nosocomial infections once a clinical ward has been contaminated. [1)]

Group A RVs are the major cause of human infections. Outbreaks with a strict seasonal winter pattern occur in temperate climates, in tropical regions infections are spread more evenly throughout the year.

After a short incubation period of 24–48 h, the onset of illness is sudden, with watery diarrhea, vomiting, and rapid dehydration. Untreated RV infection is a major cause of infantile death in developing countries. [1)]

Diagnosis may be made by rapid detection of rotavirus antigen in stool specimens.[2)] Diagnosis of rotavirus infection is based on the identification of rotavirus in feces or suspension of rectal swab collected early in the illness.[3)]

The "**ichroma™ ROTA**" is an immunoassay for the detection of group A human rotavirus in stool sample.

PRINCIPLE

The test uses a sandwich immunodetection method; Dried antibodies in the detector tube, once diluted with the diluent, bind with antigens in the sample to form antigen-antibody complexes. These complexes then migrate through the nitrocellulose matrix and are captured by another sets of immobilized antibodies on the test line.

The more antigens in the sample, the more antigen-antibody complexes, which leads to a stronger fluorescence signal. This signal then is interpreted by the reader to display rotavirus concentration in the sample.

COMPONENTS

ichroma™ ROTA consists of 'Cartridges', 'Detection Buffer Tubes', 'Diluent', 'Sample collection tubes', 'Filter caps', 'Sample swabs', 'ID chip' and an 'Instruction for use'.

- The cartridge contains a test strip, the membrane which has rotavirus antibody at the test line, with chicken IgY at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains rotavirus antibody fluorescence conjugate, anti-chicken IgY fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- The dried detection buffer is pre-dispensed in a tube. 25 detection buffer tubes are packed in an aluminum foil pouch.
- The diluent contains a detergent and bovine serum albumin (BSA) as a stabilizer and sodium azide in sodium borate buffer as a preservative. The diluent is dispensed in a vial.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detection buffer, diluent and ID chip) should agree.
- Do not interchange test components between different lots or use test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with applicable local requirement. A Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, the detection buffer and reach the room temperature by leaving them in the room for approximately 30 minutes at the least.
- **ichroma™ ROTA** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ ROTA** should be used only in conjunction with the instrument for ichroma™ tests.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer, the diluent and the sample collection tube are stable for 20 months if stored at 2-8 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.

- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-75

Components of **ichroma™ ROTA**

- Box Contains:
  - Cartridges 25
  - Detection buffers 25
  - Sample collection tubes 25
  - Filter caps 25
  - Sample swabs 25
  - Diluent vial 1
  - ID Chip 1
  - Instruction For Use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ ROTA**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ ROTA** is human feces.

- Fecal samples must be taken as soon as the symptoms (diarrhea) appear.
- Collect random samples of feces in a clean, dry container or a receptacle, making sure to exclude.
- Loosen a cap on the upper part of a sample collection tubes and remove. Use with care not to spill or splatter solution from the tube.
- Collect random samples by using a sample swab with the proper method; insert the sample swab and put the stick into the fecal samples several times (5-6 times) at different sites to get a representative sampling.

※ Fill up the groove of a sample swab with fecal samples and please check whether the quantity is too much or not.

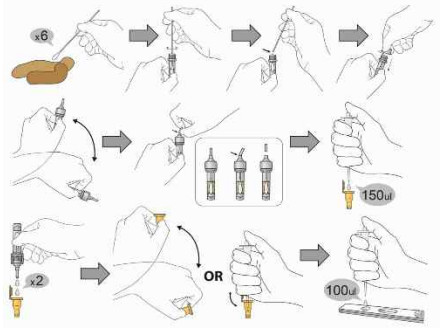
- Insert the sample swab into the sample collection tube.
- Swirl the sample swab at least 10 times. And break the sample swab at the break point and remove top portion of swab.
- Assemble filter cap on the sample collection tube.

★ Mixture may be stored for up to 3 days at 2-8 °C in a darkroom.

TEST SETUP

- Check the contents of **ichroma™ ROTA**: Sealed Cartridges, Detection Buffer Tubes, Diluent Vial, 'Sample collection tubes', 'Filter caps', 'Sample Swabs' and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip, the detection buffer as well as the diluent.
- Keep the sealed cartridge (if stored in refrigerator), the detection buffer tube and the diluent at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the Instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the Instrument for ichroma™ tests**.**
- Press the 'Select' button on the Instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE



1) Put a sample swab into the fecal sample about 5-6 times at different sites and try to avoid obtaining clumps of feces.
2) Loosen a cap on the upper part of a sample collection tube and remove.
3) Insert the sample swab into the sample collection tube.
4) Swirl the sample swab at least 10 times. And break the sample swab at the break point and remove top portion of sample swab.
5) Assemble filter cap on the sample collection tube and the filter cap end.
6) Break the break point of filter cap.
7) Open the diluent vial and transfer 150 µL of diluent using a pipette to the detection buffer tube.
8) Transfer only 2 drops (about 30 µL) of feces sample using a sample collection tube to the Detection buffer tube.
9) Mix well by pipetting 10-20 times. (The sample mixture must be used immediately.
10) Pipette out 100 µL of a sample mixture and dispense it into the DB well on the cartridge.
11) Leave the sample-loaded cartridge at room temperature for 12 minutes
    ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
12) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
13) Tab the 'Start' icon on the screen.
14) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.

Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays "IG value: Positive / Negative / Indeterminate, IM value: Positive / Negative / Indeterminate".
- Ancillary value is served in the form of a cut-off index (COI) value.

Cut-off index (COI)	Result	Note
≤ 0.9	Negative for Rotavirus	No need to additional test.
> 0.9, < 1.0	Indeterminate	Need to retest.
≥ 1.0	Positive for Rotavirus	Need to confirmation test.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ ROTA**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Sensitivity**
  - Cut-off
    The **ichroma™ ROTA** test result indicates 'positive' or 'negative' of a sample defined by the algorithm of the reader based on COI (cut-off index)

Cut-off index (COI)	Result
COI ≥ 1.0	Positive
0.90 < COI < 1.0	Indeterminate
COI ≤ 0.90	Negative

- **Analytical Specificity**
  - Cross-reactivity
    There was no false positive result from 15 species virus samples and 24 species bacteria samples containing potentially cross-reactive substances with the **ichroma™ ROTA** test. The overall specificity was 100 %.

Virus			
#1	ADV1	#9	enterovirus type 71 (ATCC VR-784)
#2	ADV2 (KBPV-VR-58)	#10	cytomegalovirus (ATCC-VR-538)
#3	ADV3 (KBPV-VR-2)	#11	poliovirus type 1 (ATCC VR-58)
#4	ADV3 (KBPV-VR-2)	#12	Coxsackie virus B type 5 (ATCC VR-1036)
#5	ADV5	#13	Coxsackie virus B type 6 (ATCC VR-1037)
#6	ADV31 (ATCC VR-1109)	#14	herpes simplex virus type 1 (ATCC-VR-733)
#7	ADV40 (ATCC VR-931)	#15	herpes simplex virus type2
#8	ADV41 (ATCC VR-930)		

Bacteria	
#1	Staphylococcus aureus (ATCC 29213)
#2	Enterococcus faecalis (ATCC 29212)
#3	Escherichia coli (ATCC 25922)
#4	Kleb-siella oxytoca (ATCC 700432)
#5	Pseudomonas aeruginosa (ATCC 27853)
#6	Neisseria gonorrheae (ATCC 27853)
#7	Aeromonas hydrophila (KCCM 32586)
#8	Enterobacter cloacae (KCCM 32586)
#9	Vibrio parahaemolyticus (KCCM11965)
#10	Salmonella group B (Clinical isolate from patient)
#11	Salmonella group C (Clinical isolate from patient)
#12	Salmonella group D (Clinical isolate from patient)
#13	Salmonella group E (Clinical isolate from patient)
#14	Shigella group D (Clinical isolate from patient)
#15	Staphylococcus epidermidis (Clinical isolate from patient)
#16	Serratia marcescens (Clinical isolate from patient)
#17	Yersinia enterocolitica (Clinical isolate from patient)
#18	Salmonella typhi (Clinical isolate from patient)
#19	Clostridium difficile (Clinical isolate from patient)
#20	Candida albicans (Clinical isolate from patient)
#21	Candida parapsilosis (Clinical isolate from patient)
#22	Campylobacter spp
#23	Proteus vulgaris
#24	Proteus mirabilis

  - Interference
    There, in test samples, are biomolecules and chemical drugs were added to the test samples at concentrations much higher than their normal physiological levels in human feces.
    ichroma™ ROTA test results did not show any significant interference with these biomolecules and chemical drugs.

Biomolecule			
#1	Bilirubin	#4	Cholesterol
#2	Hemoglobin	#5	BSA
#3	Triglycerides		
Chemical drug			
#1	cephradine	#9	metronidazole
#2	cefuroxime axetil	#10	ibuprofen
#3	Cefpodoxime proxetil	#11	acetaminophen
#4	cefixime	#12	barium sulfate
#5	tetracycline hcl	#13	DMSO
#6	levofloxacin	#14	DMF
#7	amoxicillin	#15	DDW
#8	loperamide oxide	#16	PBS

- **Precision**
  - Between Lot
    One person tested three different lots of **ichroma™ ROTA**, ten times at each concentration of the control standard.
  - Between person
    Three different persons tested one lot of **ichroma™ ROTA**, five times at each concentration of the control standard.
  - Between day
    One person tested one lot of **ichroma™ ROTA** during three days, five times at each concentration of the control standard.
  - Between site
    One person tested one lot of **ichroma™ ROTA** at three different sites, five times at each concentration of the control standard.

ROTA Cal	Between lot		Between person		Between day		Between site	
	Positive/ Number of test	Positive	Positive/ Number of test	Positive	Positive/ Number of test	Positive	Positive/ Number of test	Positive
Negative	0/10	0 %	0/5	0 %	0/5	0 %	0/5	0 %
Low	10/10	100 %	5/5	100 %	5/5	100 %	5/5	100 %
Mid	10/10	100 %	5/5	100 %	5/5	100 %	5/5	100 %

- **Comparability with reference product**

		Reference rotavirus assay		
		Positive	Negative	Total
ichroma™ ROTA	Positive	102	3	105
	Negative	1	100	101
	Total	103	103	206

  - Percent positive agreement (%) = 102/103 x 100 = 99.03 %
  - Percent negative agreement (%) = 100/103 x 100 = 97.09 %
  - Overall percent agreement (%) = (102 + 100)/206 x 100 = 98.06 %

REFERENCES

1. Rotavirus Methods and Protocols. James Gray *et al.*, Methods in Molecular Medicine., 2000, 6~7 pp.
2. Centers for Disease Control. Rotavirus| Clinical Disease Information | CDC 2014.
3. Incidence of rotavirus infection in children with gastroenteritis attending Jos university teaching hospital, Nigeria. Junaid SA *et al.*, *Virol J.*, 2011 May 16;8:233. doi: 10.1186/1743-422X-8-233. 2011.

Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

 **Boditech Med Incorporated**
43, Geodudanji 1-gil, Dongnae-myeon,
Chuncheon-si, Gang-won-do, 24398
Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

 **Obelis s.a**
Bd. Général Wahis 53,
1030 Brussels, BELGIUM
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net