MultNAT Gastrointestinal Panel
Instructions for Use

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[PRODUCT NAME]
MultNAT Gastrointestinal Panel

[SPECIFICATIONS]

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[INTENDED USE]
The MultNAT Gastrointestinal Panel is a qualitative nucleic acid-based in vitro diagnostic test intended for the qualitative detection of nucleic acids from 15 diarrhea-related pathogens from raw or unpreserved unformed (liquid or soft) stool samples. The following pathogens can be detected and differentiated using this panel: Escherichia coli 0157, Salmonella, Campylobacter, Enterotoxingenic E. coli (ETEC), Yersinia enterocolitica, Clostridium difficile Toxin A/B, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Shiga toxin-producing Escherichia coli (STEC), Shigella (combined with Enteroinvasive E. coli, EIEC), Norovirus GI/GII, Adenoviruses 40/41, and Rotavirus.
The MultNAT Gastrointestinal Panel is indicated as an aid in the diagnosis of gastrointestinal illness and results are meant to be used in conjunction with other clinical, laboratory, and epidemiological data. It cannot be used alone as the basis for diagnosis or exclusion of a case. Positive results do not rule out co-infection with organisms not included in this panel.

[MATERIALS PROVIDED]
Each panel contains sufficient reagents to test 10 samples or quality control samples.

NO.	Name	Specification	Quantity	Main Components
1	GI-Cartridge	1 test/cartridge	10	Specific primers, probes, deoxyribonucleoside triphosphate (dNTP), DNA polymerase, reverse transcriptase, uracil DNA glycosylase(UDG)
2	GI-Extraction Solution	2 mL/tube	10	Guanidine salt, magnetic beads, isopropanol
3	GI-Internal control	1 test/tube	10	Bacteriophages MS2
4	GI-Positive Control	2 mL/tube	1	Inactivated hybrid strains or pseudovirus harboring target gene fragments
5	GI-Negative Control	2 mL/tube	1	Inactivated hybrid strains or pseudovirus without target gene fragments

⚠ **Note:** The materials provided in different batches of panel cannot be used interchangeably.
Materials required but not provided: transport medium for stool sample.

[TEST PRINCIPLE]
In this assay, the cartridge is equipped with multiple hydrophobic separation layers to isolate the lysis solution, wash solution and reaction solution. The samples are chemically lysed in extraction solution at high temperature to release the nucleic acids under the heating control of the external instrument. An external magnetic field provided by the instrument allows the nucleic acid samples to pass through different layers. Finally, the nucleic acids are eluted in the cartridge legs where the amplification reaction occurs. Thus, it enables fully automatic "All-in-One" nucleic acid analysis in which the lysis, binding, washing, elution, amplification reaction and detection are completed in a closed cartridge.
This panel integrates 15 pathogen amplification systems into four channels, and amplification and detection are achieved by virtue of RT-PCR and four different fluorescent probes. The four fluorescent channels can quickly detect 15 pathogens (FAM, HEX, ROX) and internal control bacteriophages MS2 (CY5) of each reaction scheme. The internal control is used to monitor the effectiveness of nucleic acid extraction, purification, reverse transcription and amplification.
The corresponding channels and fluorescence signal of each pathogen are shown in the table below:

Channel	Pathogen	Fluorescence signal	Internal control
A	Escherichia coli (0157)	FAM	CY5
	Campylobacter	HEX	
	Salmonella (Salmo)	ROX	
B	Enterotoxingenic E. coli (ETEC)	FAM	
	Yersinia enterocolitica (YE)	HEX	
	Clostridium difficile Toxin A/B (ctdA/B)	ROX	
C	Vibrio spp.(Vc [Vibrio cholerae], VP [Vibrio parahaemolyticus], vvh [Vibrio vulnificus])	FAM	
	Shiga toxin-producing Escherichia coli (STEC)	HEX	
	Shigella (SF combined with Enteroinvasive E. coli, EIEC)	ROX	
D(Virus)	Norovirus GI/GII (NoGI/II)	FAM	
	Adenoviruses 40/41 (adv)	HEX	
	Rotavirus (RV)	ROX	

[STORAGE AND STABILITY]
1. Storage condition: store the assay at 2℃-8℃.
2. Validity period: 6 months. See label for production and expiry date.
3. Transport at ambient temperature(-25℃-30℃)within 15 days will not affect assay performance.

[SAMPLE COLLECTION AND HANDLING]
1. Sample type: stool sample; raw or unpreserved unformed (liquid or soft) stool sample
2. Sample collection
Note: Stool sample should be collected from the suspected patient with a swab and sample tube that exclusively for stool sample.
2.1 Place the swab tip into stool sample (the tip does not need to be fully immersed). Leave the swab in the tube and cap the tube.
2.2 Vibrate sample tube to mix the sample with preservation solution thoroughly.
3.Sample storage
Collected sample should be sent for testing as soon as possible. If instant testing cannot be carried out, refrigerated it at 2℃-8℃ within 3 days.
4.Sample transport
Keep the sample refrigerated at 2℃-8℃ (within 3 days) during transportation.

[APPLICABLE INSTRUMENT]
MultNAT® Molecular Diagnostic Testing System produced by Ustar Biotechnologies (Hangzhou) Ltd.

[TEST PROCEDURE]
1.Sample pretreatment
1.1 If a frozen sample is to be used, it should be completely thawed before use.
1.2 Mix the stool sample well. Centrifuge at 4,000 rpm for 30 seconds, and take liquid supernatant for testing.
Note: Avoid pipetting the residue at the bottom when take the supernatant.
2.Cartridge preparation
2.1 Put on disposable protective gloves.
2.2 Check if the cartridges is damaged. Do not use it if it is damaged.
2.3 Open the cartridge.
Pipette 2 mL of extraction solution to the internal control (tube) to dissolve the purple solid particles in the tube until they become colorless. Then, add the mixture to the cartridge. Pipette 1 mL of the sample solution and add it to the cartridge.
2.4 Cap the cartridge and shake it well.
2.5 Load the prepared cartridge into the MultNAT system.
2.6 Scan the QR code.
2.7 Enter the sample ID.
2.8 Click start to run a test.
3. External quality control testing
3.1 Pipette 2 mL of extraction solution to the internal control (tube) to dissolve the purple solid particles in the tube until they become colorless. Then, add the mixture to a cartridge.
3.2 Pipette 1 mL of positive or negative control and add it to the cartridge.
3.3 The following steps, including cartridge preparation, are the same as that [TEST PROCEDURES] 2.4-2.8.
4.Interpretation of quality control testing
4.1 The result of quality control will be saved and displayed automatically at the end of test. See [INTERPRETATION OF RESULTS].
4.2 Negative control: No visible amplification curve in FAM,HEX, and ROX, but in CY5, and CT ≤35.
4.3 Positive control: Visible amplification curves in FAM, HEX, and ROX channels and CT≤35, and visible or invisible curve in CY5 channel.
5. Cut-off value

Pathogen	Assay analysis
Escherichia coli (0157), Campylobacter (CR), Salmonella, Enterotoxingenic E. coli (ETEC), Yersinia enterocolitica (YE), Clostridium difficile Toxin A/B (cdtA/B), Vibrio spp. (Vc [Vibrio cholerae], VP [Vibrio parahaemolyticus], vvh [Vibrio vulnificus]), Shiga toxin-producing Escherichia coli (STEC), Shigella (SF combined with Enteroinvasive E. coli, EIEC), Adenoviruses 40/41 (adv), and Rotavirus (RV).	1.Visible FAM, HEX, and ROX curves are observed and CT≥40, or no curve observed but CY5 and CT < 40. In this case, result is negative. 2.Visible FAM, HEX, and ROX curves are observed and CT < 40, and CY5 curve can or cannot be observed. In this case, result is positive.
Norovirus GI/GII (NoGI/II)	1.Visible FAM curve is observed in channel D and CT≥43, or no curve observed but CY5 and CT < 40. In this case, result is negative for norovirus GI/GII. 2.Visible FAM curve is observed in channel D, CT < 43, and CY5 curve can or cannot be observed. In this case, result is positive for norovirus GI/GII.

[INTERPRETATION OF RESULTS]
The MultNAT® Molecular Diagnostic Testing System analyzes the results automatically according to the fluorescence signals measured in each channel and displays the results in the result window.

Result	Interpretation
Positive	One or more than two pathogens are detected in the sample.
Negative	None of the 15 pathogens are detected in the sample.
Invalid	It is not sure whether there is any of the 15 pathogens in the sample.
No result	Insufficient data are collected. For example, the operator stops a test in progress.

Conditions that require retesting
1.1 Reason for retesting
If any of the following results occur, please retest with a new cartridge.
-Invalid: internal control CT value not in the effective range, inappropriate handling of samples, or PCR reaction is inhibited.
-No result: insufficient data are collected. For example, the operator stops a test in progress or power outage.
1.2 Method for retesting
Use a new cartridge for "invalid" or "no result" testing (do not reuse the cartridge).
·Take out a new cartridge.
•See **[TEST PROCEDURES]**.

[LIMITATIONS]

1.Result from this panel are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms, signs, medical history, other laboratory tests and therapeutic effect.
2.Inappropriate collection, transport and treatment of samples or low viral load of pathogens may yield false negative result.
3.Other unverified interference or amplification inhibitors may yield false negative results.
4.The mutation of target nucleic acid to be tested or the sequence alteration caused by other reasons may yield false negative result.

[PERFORMANCE CHARACTERISTICS]

1.Limit of Detection (LoD)

Channel	Pathogen	Copies/mL
A	Salmonella	500
	Campylobacter	50
	Escherichia coli (0157)	500
B	Yersinia enterocolitica (YE)	500
	Clostridium difficile Toxin A(ctdA)	500
	Clostridium difficile Toxin B (ctdB)	500
	Enterotoxingenic E. coli (ETEC)	50
C	Vibrio cholerae (Vc)	500
	Vibrio parahaemolyticus (VP)	500
	Vibrio vulnificus (vvh)	500
	Shigella (SF)	500
	Shiga toxin-producing Escherichia coli (STEC)	500
	Enteroinvasive E. coli (EIEC)	500
D（Virus）	Adenoviruses 40/41 (adv)	50
	Rotavirus (RV)	500
	Norovirus GI (NoGI)	5000
	Norovirus GII (NoGII)	500

2.Cross-reactivity

The specificity of this panel was evaluated by testing common organisms in the gastrointestinal tract and feces. Three times of testing using the panel were carried out on all off-panel bacteria (1×10^6 CFU/mL) and viruses (1×10^6 copies/mL). Results were all negative without any cross reactions, indicating 100% analytical specificity of this panel. The off-panel pathogens to be tested are listed below:

Organism		
Acinetobacter baumannii	Anaerococcus prevotii	Bacteriocides fragilis
Citrobacter freundii	Clostridium sordelli	Eggerthella lenta
Enterobacter cloacae	Enterococcus casseliflavus	Enterococcus faecalis
Enterococcus faecium	Enterococcus gallinarium	Escherichia hermannii
Fusobacterium necrophorum	Helicobacter pylori	Klebsiella pneumoniae
Lactobacillus jensenii	Listeria monocytogenes	Micrococcus luteus
Morganella morganii	Peptostreptococcus anaerobiusa	Plesiomonas shigelloides
Prevotella oralis	Proteus mirabilis	Proteus vulgaris
Providencia alcalifaciens	Providencia stuartii	Pseudomonas aeruginosa
Pseudomonas fluorescens	Pseudomonas putida	Serratia marcescens
Staphylococcus aureus	Staphylococcus epidermidis	Streptococcus agalactiae
Streptococcus dysgalactiae	Streptococcus pyogenes	Astrovirus
Coxsackie virus	Echovirus	Parechovirus
Sapovirus	Candida albicans	Blastocystis hominis

3.Interfering substances

The potential interfering substances in the stool sample have correlation with the panel performance. Three times of testing on each target pathogen at test concentration were carried out, and results were not affected. The potential interfering substances are listed below:

Endogenous substances	
Name	**Test concentration**
Fecal fat/Cholesterol	5 % w/v
Hemoglobin human	12.5 % w/v
Purified Mucin protein	5 % w/v
Fatty acids/Steric acid, Palmitic acid	5 % w/v
Fecal fat/Triglyceride Mix	5 % w/v
Human Whole Blood	10 % w/v
Exogenous substances	
Name	**Test concentration**
Acetaminophen	5 % w/v
Antibiotic/Amoxicillin	5 % w/v
Ampicillin Sodium Salt	152 μmol/L
Aspartame	5 % w/v
Contrast medium/Barium sulfate	1 % w/v
Antiseptic Towelettes/ Benzalkonium Chloride in ethanol	0.2 % w/v
Bismuth (III) Subsalicylate	1 % w/v
Calcium Carbonate	5 % w/v
Hydrocortisone	50 % w/v
Ibuprofen	5 % w/v
Loperamide HCl	5 % w/v
Attapulgite	5 mg/mL
Metronidazole	5 % w/v
Nystatin	50 % w/v
Naproxen Sodium	2.2 μmol/L
Mg(OH)2, Al(OH)3 and MgCO3	5 % w/v
Polysporin/Polymyxin B Sulfate	50 % w/v
Sennaglycosides	5 % w/v
Mineral Oil	50 % w/v

4.Competitive effect study

In the testing of contrived sample with coinfection, the detection of target pathogens with the concentration at LoD was not affected by the tripling concentration of other pathogens in the same channel.

[PRECAUTIONS]

1. For research use only. Read this IFU carefully prior to use. Ustar shall not assume any responsibility for the false results and corresponding consequences due to improper handling or any problems not derived from the performance defects of the assay.

2. IVD This panel is used for in vitro diagnostic use of pathogens affecting gastrointestinal tract only. The diagnosis and treatment of patients with diarrhea and diseases associated with intestinal inflammation should be considered in combination with their symptoms, signs, and other diagnostic approaches.

3.Testing

3.1 This panel is a disposable product. Disinfect the worktable and necessary articles with 1% sodium hypochlorite solution, 75% alcohol solution or ultraviolet lamp regularly.
3.2 Follow relevant national regulations for laboratory quality control. Avoid false positive results caused by laboratory contamination.
3.3 The protective facilities used by operators, such as lab coats and gloves, should be managed separately to avoid introducing contaminants and causing test errors.
3.4 Do not squeeze the middle and lower parts of cartridge.
3.5 The extraction solution contains insoluble particles. Mix it thoroughly before adding to cartridge.
3.6 Keep intact and clean the QR code on the cap of cartridge. Do not scrawl on it or remove it.
3.7 Cartridge should be tested immediately after opening and sample loading. If it needs to be stored, the storage time shall not exceed 2 hours (shake and mix it well before loading it to the system module).
3.8 Follow the test procedures prescribed in this IFU strictly. Do not put the cartridge into the module until sample information has been input.
3.9 For quality control testing, do not add positive control into the cartridge until other materials have been added.
3.10 Centrifuging of positive control must be done before opening, and the time of opening should be shortened as much as possible.
3.11 Do not open module during testing.
3.12 Do not open cartridge after test.

4. Result viewing

Test result is stored in the system automatically at the end of test. Previous test results can be viewed in the "View" page.

5.Operation

5.1 If reagent enters eyes or mouth by mistake, or contaminates the skin, rinse with plenty of water immediately, and accept the professional medical treatment if necessary.
5.2 Before loading cartridge, make sure that the outer wall of the cartridge is free of liquid and other adherents.

6.Storage and use

6.1 Store the assay as per the conditions specified in this IFU.
6.2 Take out only the required amount of reagent for use to prevent deterioration. Maintain the rest according to the specified conditions.
6.3 Do not use the positive control (such as diluting or adding to the sample) except for the operation specified in this IFU, to avoid polluting the testing environment.
6.4 Do not use reagents that have expired.
6.5 Do not mix with other batches. Do not add or refill reagents.

6.6 Please check before use that the cartridge has no scratches or cracks.

7. 7. Disposal

7.1 Do not open any used cartridge. Dispose of the used cartridge as medical waste.

7.2 All samples and other materials should be disposed of in accordance with the [Medical Waste Management Regulations]1 after use.

[REFERENCE]

1.Medical waste management regulations: The State Council of the People's Republic of China promulgated on June 16, 2003.

[EXPLANATION OF SYMBOLS]

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
EC REP	Authorized representative in the European Community		Keep dry
	Keep away from sunlight		Do not use if package is damaged
	Date of manufacture		Biological risks
	Contains sufficient for <n> tests	REF	Catalogue number
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.		

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[INSTRUCTION VERSION AND MODIFICATION DATE]

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