Measles and Rubella virus (MeV/RV) RNA Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3278E-24, S3278E-48

Product Name

Measles and Rubella virus (MeV/RV) RNA Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification

24 tests/kit, 48 tests/kit

Intended Use

The Measles and Rubella virus (MeV/RV) RNA Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription PCR test kit intended for the qualitative detection of nucleic acid from Human Measles and Rubella virus (MeV/RV) in nasopharyngeal swabs, oropharyngeal swabs, and serum from individuals who are suspected of the MeV or RV infection. Results are for the identification of the MeV or RV RNA, and should not be used as the sole basis for patient management decisions.

The Measles and Rubella virus (MeV/RV) RNA Diagnostic Kit (PCR-Fluorescence Probing) is intended for use by professional, qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

For *in vitro* diagnostic use only. For professional use only.

Summary

The Measles and Rubella virus RNA are generally detectable in swabs or serum specimens during the acute phase of infection. Positive results are indicative of the presence of Measles and Rubella virus RNA. Clinical correlation with medical history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out viruses infection or co-infection with other bacterial. The agent detected may not be the definite cause of disease. Negative results do not preclude Measles and Rubella virus infection. Negative results must be combined with clinical observations, medical history, and epidemiological information.

Test Principle

The Measles and Rubella virus (MeV/RV) RNA Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction test kit. The kit employed speciall designed MeV/RV primers and probes sets to detect MeV and RV in respiratory tracks and serum from patients who are suspected of the MeV or RV infection. This kit is used for qualitative detection of MeV RNA and RV RNA.

A specifically designed primers and probes set is targeting the human GAPDH gene as internal control, to monitor sample collection, sample handling and qPCR process to avoid false-negative results.

Components of the Diagnostic Kit

No.	Reagent Name	Spec. & Qty.		Main Ingredients
		24 T	48 T	
1	MeV/RV PCR Mix	624 μL/tube × 1 tube	1248 μL/tube × 1 tube	Primers, Probes, dNTPs, Mg^{2+}, PCR buffer
2	MeV/RV Enzyme Mix	96 μL/tube × 1 tube	192 μL/tube × 1 tube	Taq DNA polymerase, Reverse transcriptase
3	MeV/RV Negative Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Normal Saline
4	MeV/RV Positive Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Synthetic sequences contains target of interest

Note:

1. All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kit lots.
2. Materials required but not provided: 1.5 mL DNase-free and RNase-free microcentrifuge tubes, 0.2 mL PCR tubes or strips, various models of pipettes and pipette tips (10 μL, 200 μL and 1000 μL tips with filters), microcentrifuge, vortex mixer.
3. Reagent required but not provided: Sample Release Reagent (Reference Number: S1011E) or Nucleic Acid Extraction-Purification Kit (Reference Number: S1006E) manufactured by Sansure Biotech Inc. for nucleic acid extraction.

Storage and Stability

1. This kit should be stored in its original box at -20±5°C and protected from light. The kit is valid for 12 months.
2. Please refer to the date of manufacture and expiry date printed on the outside of the box.
3. Unopened reagents are valid and stable until the expiry date.
4. Once the reagents are opened, the maximum number of freeze/thaw cycles should not exceed three.
5. The reagents keep valid and stable before the expiry date on the outer package when transporting for up to 7 days in a sealed foam box containing coolant with the temperature lower than 20°C.

Compatible Instrument

This diagnostic kit has been validated on the following Real-Time PCR instruments

1) Applied Biosystems 7500 System,
2) Thermofisher QuantStudio™ 5 Real-Time PCR System,
3) Roche LightCycler® 480 Instrument II,
4) MA-6000 Real-Time Quantitative Thermal Cycler,
5) SLAN®-96P Real-Time PCR System.

Specimen Requirements

1. Applicable specimen types: swab and serum.
2. Collection of specimens

Nasopharyngeal swab: The specimen collection tube should be pasted with the barcode first, the nasopharyngeal swab should be collected within 3 days after the onset of the disease as quick as possible. Use swab to measure the length between apex nasi and earlobe, mark the distance with finger. Insert the swab into the nasal cavity in direction of perpendicular to the nose (face). The swab should be inserted at least half of the length from the earlobe to the apex nasi. Hold the swab stops in the nasal for 15~30 s, gently rotate 3~5 times, quickly put swab into specimen collection tube containing 2 mL Lysis Buffer (same as Lysis Buffer in the Sample Release Reagent) or Sample Storage Reagent containing RNasin. Insert the swab into the specimen collection tube, break the swab rod at the breaking point near the top, tighten tube cap and seal with sealing film.

Oropharyngeal swab: Use a sterilised flocking swab for sampling. Gently wipe the posterior pharyngeal wall, do not touch the tongue. Insert the rod into a sample collection tube. Break the swab at the breaking point near the top. Tighten tube cap and seal with sealing film.

Serum: Use a sterilised syringe to draw 2 mL of venous blood into a sterilised tube, incubate under room temperature for up to 4 hours to separate serum and blood cells, or centrifuge it at 1600 rpm for 5 minute. Transfer the serum to a 1.5 mL sterilised tube for later use.

3. Storage and delivery of specimens:

Specimens to be tested can be immediately processed, specimens to be tested within 24 hours can be stored at 4°C. Specimens that cannot be detected within 24 hours should be stored at -70°C or below (in the absence of -70°C storage conditions, specimens to be tested can be stored at -20°C for 10 days, nucleic acid can be stored at -20±5°C for 15 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen container with ice or in a sealed foam box with ice packs.

Test Method

Please process according to the following steps for Applied Biosystems 7500 System, Thermofisher QuantStudio™ 5 Real-Time PCR System, Roche LightCycler® 480 Instrument II, MA-6000 Real-Time Quantitative Thermal Cycler, SLAN®-96P Real-Time PCR System

1. Preparation of reagent (performed at "reagent preparation room")

1.1 Take out each component from the diagnostic kit and place them at room temperature. Equilibrating all reagents to room temperature, vortex follow by a short spin for later use.

1.2 Prepare the MeV/RV PCR Master Mix according to following table. The volume required is based on the total number of specimens, plus a MeV/RV Positive Control and a MeV/RV Negative Control. Mix thoroughly then centrifuge it for later use. The remaining reagent must be stored at -20°C immediately.

	1 sample	24 samples	48 samples
MeV/RV PCR Mix	26 μL	624 μL	1248 μL
MeV/RV Enzyme Mix	4 μL	96 μL	192 μL

Note: The above configuration is for reference only.

2. Processing and loading of specimens (performed at "specimen processing room")

2.1 Use Sample Release Reagent (Reference Number: S1011E) or Nucleic Acid Extraction-Purification Kit (Reference Number: S1006E) manufactured by Sansure Biotech Inc. to extract the nucleic acid according to corresponding manual.

2.2 Add 20 μL of the extracted RNA, Negative Control, Positive Control, to the PCR tubes in the following order: MeV/RV Negative Control, patient specimens, and MeV/RV Positive Control.

2.3 Add 30 μL of the MeV/RV PCR Master Mix into each well. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into the PCR system.

3. PCR Amplification (Refer to user manual of each instrument to adjust the settings.)

3.1 Place PCR tubes into the specimen wells of the amplification equipment. Set up the MeV/RV Negative Control, specimens and MeV/RV Positive Control to be tested in order and input specimen name.

3.2 Select PCR test channel:

1) Select FAM channel (Reporter: FAM, Quencher: None) to test MeV RNA, 2) Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None) to test Internal Control, 3) Select ROX channel (Reporter: ROX, Quencher: None) to test RV RNA, 4) Set passive reference: none. Set Sample Volume: 50.

3.3 Set cycle parameters

	Steps	Temperature	Time	Cycles
1	Reverse transcription	50°C	30 min.	1
2	cDNA pre-denaturation	95°C	1 min.	1
3	Denaturation	95°C	15 sec.	45
	Annealing, extension and fluorescence collection	60°C	30 sec*.	
4	Device cooling	25°C	10 sec.	1

When the settings are completed, save the settings and carry out the reaction procedure. (*Note: Due to the ABI 7500 instrument, it cannot be set to 30 seconds, but can be set to 31 seconds or 32 seconds.)

4. Result Analysis (Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Analyze amplification curve of target of detection and internal control. Adjust Start, End and Threshold values of Baseline of the graph according to analysis result (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis, make sure each parameter satisfies the requirements given in "5. Quality Control". Go to "Plate" window to record qualitative results.

5. Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The end user is required to review fluorescent curves before final interpretation. All positive curves should be typical S-shaped amplification curves or without plateau for weakly positive samples.

MeV/RV PCR Positive Control			MeV/RV PCR Negative Control			Results	Actions
MeV (FAM)	RV (ROX)	IC (HEX/VIC)	MeV (FAM)	RV (ROX)	IC (HEX/VIC)		
+	+	+	-	-	-	valid	Continue to result interpretation
Either/Both of them shows negative			No required			invalid	RT-qPCR failed, re-run
No required			Either/Both of them shows positive				Extraction or RT-qPCR contaminated, re-run

Note: Ct >40 or display No Ct is negative (-), Ct ≤35 is positive (+). If the retest is contaminated, please perform a decontamination treatment.

Reference Range

Through the research on reference values, the Ct reference value of target gene is determined to be 40, the Ct reference value of internal control is determined to be 40.

Explanation of Detection Result

1. For FAM channel with Ct ≤40, and internal control has Ct ≤40, it should be reported to MeV RNA is positive. For FAM channel Ct >40 or No Ct, and internal control is detected Ct ≤40, it should be reported to MeV RNA is negative.
2. For ROX channel with Ct ≤40, and internal control hasCt ≤40, it should be reported to RV RNA is positive. For ROX channel Ct >40 or No Ct, and internal control is detected Ct ≤40, it should be reported to RV RNA is negative.
3. If the internal control detected with Ct >40 or No Ct, then the specimen's detection result is invalid. An investigation should be performed to find out reasons and then retest the specimens. (If repeated tests still produce invalid results, please contact Sansure Biotech Inc..)

Limitations of Detection Method

1. False negative can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.
2. Mutations occur in the target sequences of the MeV/RV may lead to false negative results.
3. Improper reagent storage may lead to false negative results.
4. Use of this assay is limited to personnel who are trained in the procedure.
5. Test results of the diagnostic kit can only be used as an aid in clinical diagnosis. Symptoms and physical signs, medical history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered for the clinical diagnosis and treatment.
6. Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

Product Performance Index

1. Specificity

The Measles and Rubella virus (MeV/RV) RNA Diagnostic Kit (PCR-Fluorescence Probing) has no cross-reactions with Varicella-zoster virus, Mumps, Hepatitis B virus, Hepatitis C virus, the human immunodeficiency viruses, *Japanese Encephalitis*, Dengue fever virus.

2. Limit of detection

The limit of detection of this kit is 600 copies/mL.

3. Precision

The coefficient of variation (CV%) of Ct of the precision is ≤5%.

4. Possible interfering substances in specimens

Bovine submaxillary gland, type I-S (≤20 μg/mL), Nasal sprays or drops (≤100 μg/mL), Nasal corticosteroids (≤50 μg/mL), Hemoglobin (≤2 mg/dL), Total Bilirubin (≤28 mg/dL), Triglyceride (≤3 g/dL), Blood (human, 5% (v/v)), Penicillin (≤20 μg/mL), Vitamin C (≤30 μg/mL), Dexamethasone (≤0.3 μg/mL) Ribavirin (≤300 μg/mL) have no significant interference with the detection results of the kit.

Precautions

1. For *in vitro* diagnostic use only (IVD).
2. Follow standard precautions. All specimens and positive controls should be considered potentially infectious and handled accordingly.
3. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
4. Please read the package insert carefully prior to operation. The Measles and Rubella virus RNA (MeV/RV) Diagnostic Kit (PCR-Fluorescence Probing) is only for emergency use as an *in vitro* diagnostic (IVD) test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
5. Separate laboratory rooms, dedicated to perform predefined procedures of the assay, are required. a) 1st room: Preparation Room—Prepare testing reagent; b) 2nd Room: specimen processing—Process the specimen and controls; c) 3rd: Amplification Room—PCR conducted.
6. All specimens for detection should be handled as if infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.

Bibliography

1. Bitsko R H, Cortese M M, GH Dayan, et al. Detection of RNA of mumps virus during an outbreak in a population with a high level of measles, mumps, and rubella vaccine coverage. 2018.
2. Ammour Y, Faizuloev E, Borisova T, et al. Quantification of measles, mumps and rubella viruses using real-time quantitative TaqMan-based RT-PCR assay [J]. Journal of Virological Methods, 2013, 187(1):57-64.

Symbols

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device		Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
LOT	Lot Number	REF	Reference Number
Σ	Number of Tests	EC REP	Authorized representative in the European Community
	Any warnings and /or precautions to take	CE	This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices.

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