AMD Ltd Measles virus (MeV) RT-qPCR Detection Kit

CE IVD

REF **KD919189-100**

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

The assay is an *in-vitro* Real-Time RT-PCR assay for the qualitative detection of the Measles virus using the TaqMan® detection method in a highly sensitive one step RT-qPCR kit.

For use in *In Vitro* Diagnostics.

Overview

Measles remains one of the most important causes of child morbidity and mortality worldwide. It is an acute viral, infectious disease, caused by the Measles virus, that is spread by coughing, sneezing, close personal contact or direct contact with infected nasal or throat secretions. Common complications include diarrhoea, middle ear infections and pneumonia although, less commonly, the virus can also cause seizures, blindness or inflammation of the brain. The virus remains active and contagious in the air or on infected surfaces for up to 2 hours, and Measles outbreaks can result in epidemics that cause many deaths, especially among young, malnourished children.

Principle of the Test

This kit is designed for the detection of MeV RNA using hydrolysis probe RT-qPCR. Reverse transcription of RNA to produce cDNA is followed by amplification of the MeV N gene, which is labelled with fluorescent reporter dyes. Detection then occurs by the hydrolysis probe method of qPCR.

In the reverse-transcription step, the partially denatured RNA secondary structure facilitates the synthesis of a single strand of DNA that is complementary (cDNA) to the sequence of the RNA used in the reaction. This can then be amplified by a DNA polymerase, generating double-stranded cDNA. Following the RT reaction, the forward and reverse primers hybridize to the target double stranded cDNA. A fluorogenic probe is included in the same reaction mixture which consists of an oligonucleotide labelled with a 5'-reporter dye and a downstream 3'-quencher. As the new target cDNA strand is synthesised, the probe is hydrolysed by the 5' to 3' exonuclease activity of Taq polymerase, leading to a physical separation of the fluorescent reporter from the quencher (Figure 1). The detector is then able to record this as an incremental increase in fluorescence signal from that well.

The assay consists of pairs of forward and reverse primers, and probes labelled with 5' fluorescent reporter dyes and a 3' quenchers. An internal positive control assay is also provided in order to assess the quality of the extracted cDNA and the effect of any PCR inhibitors that may be present. These assays are also multiplexed in a ready-to-use PCR master mix which utilises hot start technology, thus minimising non-specific reactions and ensuring maximum sensitivity.

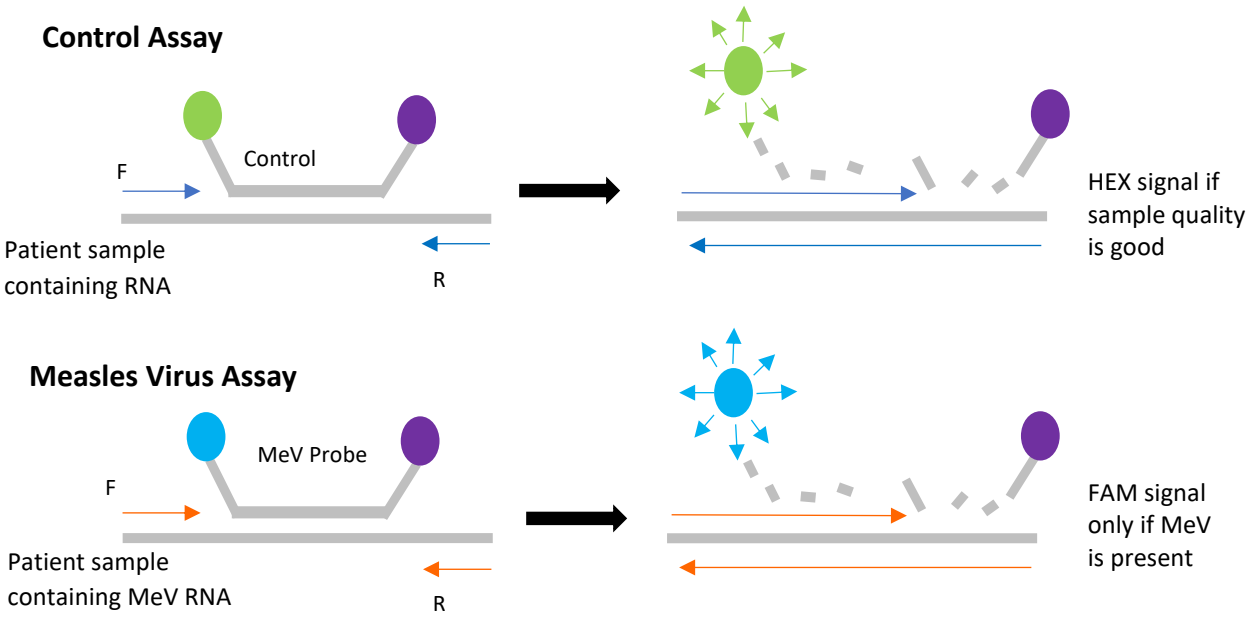

Figure 1. The principle of RT-qPCR with hydrolysis probe detection for identifying the presence Measles. The control assay in the master mix will produce a HEX signal if the cDNA quality is acceptable. The Measles assay will produce a FAM signal, if there is Measles present, however if it is not present, no FAM signal will be detected. Due to assay competition, the HEX signal may be reduced or absent when the FAM signal is strong.

Materials Provided

Kit Contents

Item	KD919189-100
AMD MeV qPCR Master Mix	2 x 1ml
MeV RT enzyme mix	1 x 0.1 ml
MeV positive control	1 x 0.05 ml
Nuclease-free water	1 x 1 ml

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -10 °C and -30 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality. It is recommended that the master mix is aliquoted to avoid this. Avoid exposure to light. Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.

Materials and Equipment Required (not provided)

RNA Extraction: AMD recommends using the Luco-Viral NA Extraction Kit to isolate RNA from the samples. Other leading kits, such as the QIAamp MinElute Virus Spin Kit (Qiagen) or in-house methods are acceptable for use with this diagnostic kit, providing that it has been validated prior to use on patient samples.

PCR Instrument: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes. It is also compatible with low, high and no ROX instruments.

Consumables: AMD manufactures high quality nuclease and pyrogen free PCR plastic ware suitable for use with this kit. Use of other manufacturers' consumables is also acceptable.

Other Laboratory Equipment: Vortex, micro centrifuge, micro pipettes and tips, microfuge tube rack, PCR tube/plate rack, spectrophotometer.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Discard sample and assay waste according to your local safety regulations. It is essential to follow the instructions in this manual precisely, to ensure accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD Zena Max Measles RT-qPCR Kit.

Instrument compatibility

AMD Measles Virus (MeV) RT-qPCR Kit is compatible with the most common Real Time qPCR equipment with the capability of detecting FAM and HEX fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Aglient Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Sample collection, Storage and Transport

The sample for AMD Measles Virus (MeV) RT-qPCR Kit should be collected from either a nasopharyngeal swab, throat swab, or urine specimen as well as a blood specimen, although Nasopharyngeal or throat swabs are preferred. Please ensure that the sample is stored correctly and kept away from any contamination. Aliquot and store the samples at -20^{0}C or -80^{0}C immediately if they are not to be used within this time-period. Freeze thawing may compromise the test results. Ensure that samples are stored correctly and kept away from any contamination.

For transportation, the samples should be placed in a shatterproof transport container to avoid the potential danger of infection due to sample leakage. Transport samples following the local and national instructions for the transport of pathogenic material, by courier, if possible, at a temperature of 2-8^{0}C and no longer than 6 hours following collection.

Assay Procedure

Sample preparation

For optimal results use the Luco-Viral NA Extraction Kit to isolate RNA from the samples. The resulting nucleic acid will be a mixture of DNA and RNA. It is important to ensure that all samples are kept free from any contamination and correct storage procedures are followed to ensure there is no damage to the RNA. Quantify the RNA using a spectrophotometer and dilute to 3-5ng/µl in nuclease free water or nuclease free TE buffer pH8.0. Store the RNA at 2-8°C for up to 24 hours, then at -20°C for longer term storage to ensure there is no damage to the RNA.

qPCR Set Up

1. Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
2. Set up the reactions on a cool block or ice using the table below, ensuring to include duplicate reactions for all samples and controls. If preferred, make a mix of assay and reverse enzyme mix and aliquot 15µl into the PCR tubes/plate.

Product	Volume x1	Volume x 10
MeV M. mix	19µl	190µl
MeV RT Enzyme Mix	1µl	10µl
RNA sample/Control	5µl	5µl*

3. Add the RNA samples and the Measles control to the PCR tubes/plate. Also add 5µl nuclease free water in place of the RNA as a No Template Control (NTC).
4. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present.
5. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile: set the qPCR instrument to the stages below

Stage/Step	Temperature	Time
Stage 1: Step 1	40°C	5mins
Stage 1: Step 2	95°C	5mins
40 Cycles		
Stage 2: Step 1	95°C	10secs
Stage 2: Step 2	58°C	30secs

*Data collection step in FAM (diagnostic assay) and HEX (internal control assay) channels.

6. When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

7. Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Interpretation of Results

The results of this qualitative assay should be interpreted as follows, using Table 1 as a quick reference guide:

- The internal control assay signal in the HEX (yellow) channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay (FAM) signal is strong.
- If there is a signal in the FAM (green) channel, with or without a HEX signal, the sample is **positive** for Measles.
- If there is a HEX signal but no FAM signal, the sample is **negative** for Measles.
- If there is no signal in either channel, the result is **inconclusive**.

Result		Interpretation
HEX	**FAM**	
Positive	Positive	Positive for MeV
No Cq	Positive	Positive for MeV
Positive	No Cq	Negative for MeV
No Cq	No Cq	Inconclusive

Table 1. Interpretation of the results obtained from the Zena Max Measles Virus (MeV) RT-qPCR Kit.

Technical Specifications

Quality: All AMD kits are manufactured under high quality standard methods and unique precision.

Sensitivity: AMD Nasopharyngeal or throat swabs Measles kits are very sensitive, reaching up to 10 copy/rxn "rxn volume 25µl" under our validation methods and devices.

Specificity: AMD Measles Virus (MeV) kits are very specific with up to 100% for Measles under our validation methods and devices.

Product Limitations

The kit is for *in vitro* diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally mutations may arise in the genomic region targeted by the primers and probes of this this assay, leading to reduction in performance or failure of the assay. The assay design and efficacy are reviewed periodically.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA and population genotyping studies to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity.

References

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Feldman, A.G., O'Leary, S.T. and Danziger-Isakov, L., 2021. The risk of resurgence in vaccine-preventable infections due to coronavirus disease 2019—related gaps in immunization. *Clinical Infectious Diseases*, *73*(10), pp.1920-1923.

Griffin, D.E., Lin, W.H. and Pan, C.H., 2012. Measles virus, immune control, and persistence. *FEMS microbiology reviews*, *36*(3), pp.649-662.

Contact

If you have any queries, comments or complaints please refer to our website at:

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Harmonised Symbols

The following is a key of all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	Name and address of EU Representative	Advena Ltd Tower Business Centre, 2nd Floor, Tower Street, Swatar BKR 4013 Malta
	UDI-DI number for the product given	Basic: 506105998MEVHZ UDI-DI: (01)05061059980489 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD919189-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC