Promega

Product Application

Purification of Viral RNA following the CDC 2019-nCoV EUA Extraction Protocol using the Maxwell® RSC 48 Instrument

Purify viral RNA from various matrices using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit with the Maxwell® RSC 48 Instrument following the recommended nucleic acid extraction procedures in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Emergency Use Authorization (EUA)[1].

Kit: Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)

Analyses: RT-qPCR for detection of Respiratory Syncytial Virus (RSV), Influenza A (Flu A) and MS2.

Sample Type(s): --Samples collected in Universal Transport Medium (UTM®) for Virus, Viral Transport Medium (VTM)[2], or PrimeStore® MTM
e.g. nasopharyngeal swabs
--Saliva collected in the Spectrum Solutions SDNA-1000 Saliva Collection Device

Input: 120µl

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM420, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

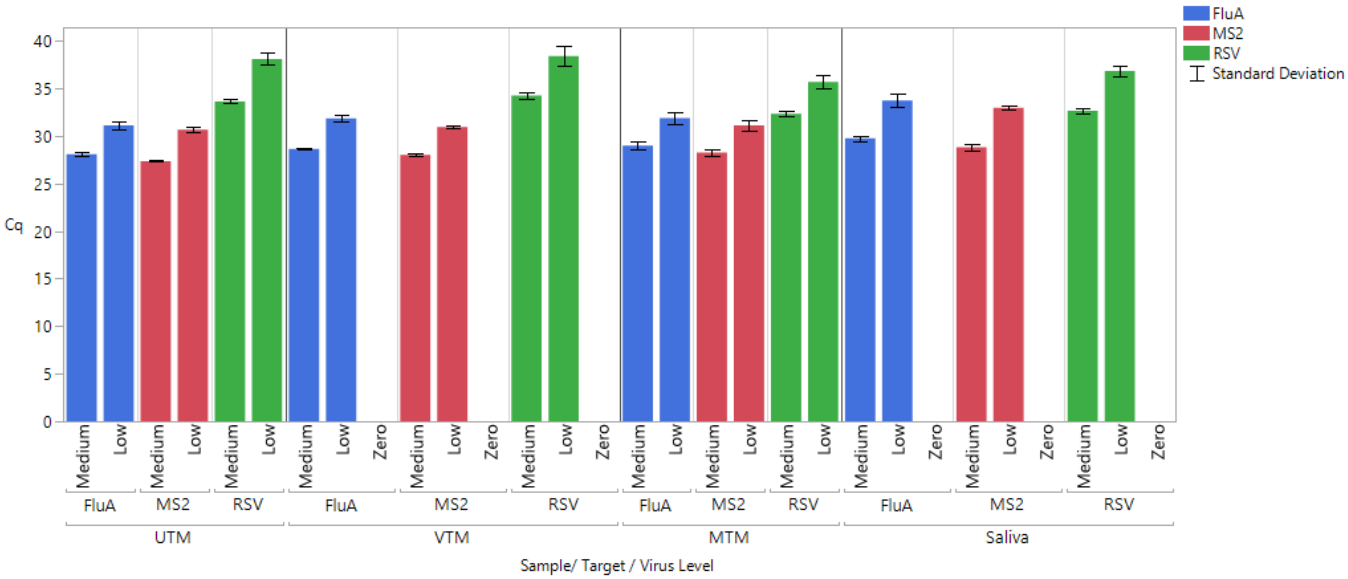
Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Heat block set to 56°C

Protocol:

1. Add 300µl Lysis Buffer and 30µl Proteinase K to a 1.5ml tube. Alternatively, prepare a master mix of Lysis Buffer and Proteinase K for all samples immediately before use, and add 330µl of the master mix to a 1.5ml tube.
2. Add 120µl of sample to the 1.5ml tube.
3. Vortex for 10 seconds.
4. Incubate samples at 56°C for 10 minutes.
5. Meanwhile, prepare cartridges as indicated in the technical manual (TM420).
    a. Add 75µl of Nuclease-Free Water to the provided elution tubes.
6. Transfer the entire lysate (450µl) to well #1.
7. On the Maxwell® RSC 48 Instrument, select the Maxwell® RSC Viral Total Nucleic Acid run method, place the prepared deck tray in instrument, and start the method.

Results:



Detection of RSV, Influenza A and MS2 RNA extracted from different sample types. Inoculated transport media and stabilized saliva samples were spiked with RSV A and Influenza A (H1N1) virus reconstituted from a Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N) and MS2 bacterophage. The medium virus sample contains approximately 1.2 x 10^4 copies of RSV A, 5.4 x 10^4 copies of Influenza A and 1.2 x 10^7 PFU per sample of MS2 bacteriophage. Low virus sample is a 1:10 dilution of the medium virus sample. Nucleic acid was purified from 120µl of the spiked sample with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC 48 Instrument as described above. Unspiked samples (zero) were included for VTM and saliva samples. Following nucleic acid purification, presence of RSV A, Influenza A and MS2 RNA was detected by RT-qPCR using GoTaq® 1-Step Probe qPCR System (Cat.# A6121). Each RSV A and Influenza A reaction contained 5µl of eluate with 12.5µl of the GoTaq® Probe qPCR Master Mix with dUTP, 0.5µl of GoScript™ RT Mix for 1-Step RT-qPCR, 1000nM forward and reverse primers and 200nM probe for RSV[3] or Influenza A[4], and Nuclease-Free Water added to a final volume of 25µl. 1-step RT-qPCR thermal cycling was as follows: reverse transcription at 45°C for 15 minutes, hot-start activation at 95°C for 2 minutes, and then 45 cycles of denaturation at 95°C for 3 seconds and annealing/extension at 55°C for 30 seconds, with signal acquisition during the annealing/extension stage of cycling. MS2 RNA was amplified using the same conditions, except that 900nM forward and reverse primers with 250nM probe were used in the reaction mix, and the anneal/extension step at 60°C for 1 minute. Data represent the average of triplicate purifications amplified in duplicate. No amplification was observed for the unspiked (zero) samples. Error bars indicate standard deviation of n=6.

References:

1. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel EUA. CDC-006-00019, Revision: 05, Effective 07/13/2020.
2. Centers for Disease Control and Prevention. (2020). Preparation of Viral Transport Medium. SOP# DSR-052-01. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf.
3. Fry, A.M., *et al.*, (2010) The Burden of Hospitalized Lower Respiratory Tract Infection due to Respiratory Syncytial Virus in Rural Thailand, *PLoS One*. *5*, e15098.
4. Selvaraju, S.B., *et al.*, (2010). Evaluation of Three Influenza A and B Real-Time Reverse Transcription-PCR Assays and a New 2009 H1N1 Assay for Detection of Influenza Viruses, *Journal of Clinical Microbiology. 48*, 3870-3875.