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Evaluation of CITOSWAB® Virus Collection and Transport Kits by Molecular Techniques

Abstract

CITOSWAB® Virus Collection and Transport kit is a ready-to-use system used for the collection and transport of viruses obtained from clinical specimens. Each Collection and Transport Kit comprises a sterile peel pouch containing a swab used to collect the sample, and a transport tube containing medium into which the swab is placed after sampling used to transport the sample. The purpose of this evaluation is to establish the suitability and stability of this kit, to enable the recovery and detection of SARS-Cov-2 (RNA) using commercial molecular techniques.

Introduction

Molecular techniques have become the corner stone of infectious disease diagnosis in the modern clinical microbiology laboratory. It is generally known that the Real-time RT PCR (rRT-PCR) assays can be used for the in vitro qualitative detection of SARS-Cov-2 in respiratory specimens and sera. Therefore, it is essential that any clinical materials for sampling and transporting must be biocompatible and free of inhibitors to allow for the recovery and detection of microbiological nucleic acids in the laboratory analysis. CITOSWAB® Virus Collection and Transport kit was tested against SARS-Cov-2 by RT-PCR.

Methods

Swabs provided as part of the CITOSWAB® Virus Collection and Transport kit, were inoculated in 100µl of the appropriate dilution of SARS-Cov-2 and immediately placed into the preservation medium (VTM), and Vortexed for two minutes. These swabs were inactivated using manufacturers lysis buffer and spiked with an internal control target prior to nucleic acid extraction. Extraction was performed by Nucleic Acid (RNA) Extraction Kit and the resulting eluates were prepared by SARS-CoV-2 Fluorescent PCR kit, and performed RT-PCR using the Applied Biosystems 7500 Real-Time PCR Systems. This is based on taqman hydrolysis chemistry, the three genes of ORF1ab, E an N gene in SARS-Cov-2 RNA would be simultaneously detected, and the positive data is represented as CT values.

The interpretation of CT value of the target gene is shown in Table 1.

Table 1 The interpretation of CT value of the target gene

Result of judgement	Negative	Positive
ORF 1ab	> 38 or no CT value	≤38
E gene	> 37 or no CT value	≤37
N gene	> 38 or no CT value	≤38
Internal Control	≤38	/

Based on the above test results, the sample interpretation is listed in Table 2.

Table 2 The interpretation of CT value of the sample

Test Result	Result interpretation
ORF1ab, N gene and E gene are all positive	SARS-CoV-2 positive
ORF1ab positive and N gene positive	
ORF1ab positive and E gene positive	
Only ORF1ab gene positive	Repeat the test. If it is still positive, it will be interpreted as SARS-CoV-2 positive
Only ORF1ab gene negative	Repeat the test. If ORF1ab, and N gene and/or E gene are positive, interpret the result as SARS-CoV-2 positive; Otherwise interpret the result as suspect who need to retested the other time.
ORF1ab negative and N gene positive	
ORF1ab negative and E gene positive	
ORF1ab, N gene and E gene are all negative	SARS-CoV-2 negative

Results

The result was displayed in table 3 below. These CT values of ORF1ab, N gene and E gene in all tested swabs were less than 37, which interpreted as SARS-CoV-2 positive.

Table 3: SARS-Cov-2 virus PCR results, CT Values

Target	CT Value 1	CT Value 2	CT Value 3	CT Value 4	Result
ORF 1ab gene	32.28	33.35	30.54	27.00	Positive
E gene	31.40	32.91	29.80	26.60	Positive
N gene	30.34	31.29	28.52	25.40	Positive
Internal Control	31.89	32.22	32.86	32.77	Negative

Discussion

The target nucleic acid was successfully detected with no inhibition in RT-PCR assay. The CITOSWAB® Virus Collection and Transport kit is an efficient and stable system for the transport and preservation of SARS-Cov-2 RNA.