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RE: Summary Validation Data of 3D Med ANDis + 3D Med Extraction + 3D Med PCR

SUMMARY

All RNA extraction for the validation 3DMed 2019-nCoV RT-qPCR Detection kit were performed by using ANDiS Viral RNA Auto Extraction and Purification Kit on the 3DMed ANDiS 350 Automated Nucleic Acids Extraction System. Real-time PCR was performed on Applied Biosystems Quant Studio 12k Flex.

Limit of Detection (LoD):

A two-phase approach was used to determine LoD. A series of serial dilutions were performed using NR-52286 control material in molecular grade water. The stock concentration of NR-52286 is 1.16 x 10^9 copies/mL. Molecular grade water was spiked with NR-52286 at 11600 copies/uL, 1160 copies/uL, 116 copies/uL, 11.6 copies/ul, 1.16 copies/uL, 0.232 copies/uL, 0.116 copies/uL, 0.0116 copies/uL in triplicates. The prepared dilutions were extracted. Extracted RNA was further tested using 3DMed 2019-nCoV RT-qPCR Detection kit on Applied Biosystems Quant Studio 12k Flex. This test provided the preliminary LoD (1xLoD) highlighted in red in the table below. Internal control was detected for all samples.

The preliminary LoD was determined to be at 1:5000000 dilution (highlighted in red) with 0.232 copies/uL or **5.8 copies/reaction. This was referred to as 1xLoD.**

The LoD was confirmed by spiking 20 replicates of pooled NP swab remnant specimens with control NR-52286 at 1xLoD. The LoD studies determines the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positives) replicates (or 19/20 replicates) tested positive using 3DMed 2019-nCoV RT-qPCR Detection kit**. For this test, 95% of the replicates (19/20 replicates) spiked at 1xLoD were tested positive for both targets N and E gene, and ORF1 ab gene.** Internal control was detected for all samples.

Sensitivity/Specificity

Sensitivity

Sensitivity was tested by spiking confirmed negative NP swab specimen pool with compounds expected to be found in the nasal passages of sick people -Vaseline, KY Jelly, and Nasal Spray. These specimens were also spiked with control NR-52286 at a dilution of 116 copies/uL (1:10000 dilution of NR-52286). These spiked samples were extracted and tested by 3DMed 2019-nCoV RT-qPCR Detection kit. They were positive for E/N gene, ORF1 ab and IC targets, indicating that the presence of the interfering substance does not affect the performance of the 3DMed 2019-nCoV RT-qPCR Detection kit and the extraction kit.

Inclusivity (Analytical Sensitivity)

Detailed analysis of analytical sensitivity was performed by 3D Biomedicine Science & Technology Co., Ltd. In summary, sequence alignment was performed with the oligonucleotide primer and probe sequences of the 3DMed 2019-nCoV RT-qPCR Detection Kit with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 20, 2020 to demonstrate the predicted inclusivity of the 3DMed 2019-nCoV RT-qPCR Detection Kit. All the alignments show 100% identity of the 2019-nCoV Assay to the available 2019-nCoV sequences. The alignment of the 2019-nCoV Assay includes additional sequences for SARS-CoV, MERS-CoV, and other Bat coronaviruses to show that other than SARS viruses, the alignment shows low identities and would not predict significant reactivity.

The inclusivity study was conducted *in silico* by mapping the assays to all analyzed SARS-CoV-2 sequences in NCBI and GISAID database as March 15, 2020. The mapping results are concluded as following and the data is available per request.

- Primer and probe sequences for 2019-nCoV ORF 1ab assay had 100% homology to all analyzed SARS-CoV-2 sequences.
- Primer and probe sequences for 2019-nCoV E gene assay had 100% homology to all analyzed SARS-CoV-2 sequences, with four exceptions such as EPI_ISL_408487 (hCoV-19/He0n/IVDC-HeN-002/2020) and EPI_ISL_408486 (hCoV-19/France/RA739/2020) showed no alignment with primer probe in 2019-nCoV E gene assay, The potential root cause may be the quality or the lengths of the reference sequences in the database. In addition, EPI_ISL-413752 (hCoV-19/Chi0/WF0023/202) showed 4 mismatched in the probe and no alignment with E gene reverse primer, and EPI_ISL_414015 (hCoV-19/Brazil/SPBR-06/2020) showed 61% homology with forward primer of E gene assay. These mismatches indicated that a potential false negative result will be reported for a specimen containing the sequence as EPI_ISL-413752 or EPI- ISL-414015.
- The mapping results for primer and probe in N gene assay showed less than 90% homology with multiple strains of SARS-CoV-2 sequence, therefore, a potential false negative result will be reported.

Cross Reactivity/Analytical Specificity

To test specificity of the 3DMed primer and probe sequences, confirmed negative RNA extracts were pooled and spiked with control obtained from IDT for the following organisms:

1. SARS (Catalog# 10006620)
2. MERS (Catalog# 10006619)

SARS and MERS were not detected by any targets of 3DMed 2019-nCoV RT-qPCR Detection kit. IC was detected for all samples.

Detailed analysis of analytical specificity was performed by 3D Biomedicine Science & Technology Co., Ltd. As per the *in silico* analysis study of 3DMed 2019-nCoV RT-qPCR Detection kit, the 2019-nCoV ORF1ab assay, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive RT-qPCR results. The 2019-nCoV E and N assays were designed for universal detection of 2019-nCoV, human SARS coronavirus and bat SARS coronavirus.

Due to the availability of extensive data and wet lab analysis testing done by 3D Biomedicine Science & Technology Co., Ltd, and back-ordered target organisms, extensive cross-reactivity/specificity studies were not performed at Provista Diagnostics.

Reproducibility/Precision

Intra-assay Precision

All preliminary LoD specimens were run in triplicates on Run-1. Results showed 100% concordance at 1xLoD (highlighted in red) and above 1x LoD.

Inter-assay Precision

Preliminary LoD was confirmed by spiking 20 replicates of pooled negative clinical specimens with control NR-52286 at 1xLoD and testing on 3DMed 2019-nCoV RT-qPCR Detection kit. The Results showed 100% concordance at 1xLoD for all replicates of Run-2 showing intra-assay precision. Results also showed 100% concordance for 3 replicates at 1xLoD from Run-1 and 20 replicates at 1xLoD from Run-2. This demonstrates inter-assay precision.

Clinical Agreement/Accuracy

30 clinically derived specimens (CDS) were obtained from ISpecimen Inc. These were confirmed to be negative nasopharyngeal swabs remnants. RNA was extracted from the 30 specimens followed by testing the specimens on 3DMed 2019-nCoV RT-qPCR Detection kit.

All 30 negative specimens were negative for all targets for SARS-CoV-2 detected by the kit. IC was detected for all 30 targets.

34 confirmed positive samples were obtained from iSpecimen Inc. 33 samples were positive for all SARS-CoV-2 targets and IC.

1 sample (P-9) was not detected for either N/E- gene or ORF1 ab target. P-9 sample was also extracted using Qiagen QIAamp Viral RNA Mini Kit (Cat# 52906) and Omega Biotek Mag-Bind® Viral DNA/RNA 96 Kit (Cat# M6246-03) and tested on 3DMed 2019-nCoV RT-qPCR Detection kit. P-9 remained undetected for all SARS-CoV-2 targets. The RNA extracted for P-9 using all 3 extraction methods was also tested on the validated Gnomegen COVID-19 RT-qPCR detection kit (Cat# CV0303) and the CDC 2019-Novel Coronavirus (2019-nCoV) Real Time RT-PCR Diagnostic Panel (Cat#269183028). The sample remained undetected for all targets. Resultantly, P-9 was excluded from the validation. **100% accuracy was observed for both positive and negative specimens.**