Development of ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 for Sensitive and Accurate Detection of the SARS-CoV-2 virus

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1. Introduction

The novel coronavirus SARS-CoV-2, responsible for COVID-19 pandemic, has infected over 40 million cases worldwide with death toll of over 1.1 million. These numbers are expected to continue increasing with winter approaching the northern hemisphere and as new infection waves emerge, thus leading to significant impact on health care and social systems. Thus, there is an urgent need for a fast, high-throughput and accurate diagnostic method to aid timely treatment for those infected and to prevent the spread of the virus. Here, we developed an automated workflow for the qualitative detection of SARS-CoV-2 RNA in suspected COVID-19 positive patients which allows for minimal hands-on and high throughput screening of patients.

2. Methodology (Workflow)

The ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 is a real-time RT-PCR-based in vitro diagnostic test intended for qualitative detection of SARS-COV-2 RNA in clinical specimens (nasopharyngeal and oropharyngeal swabs) on an automated workflow as shown in Figure 1.

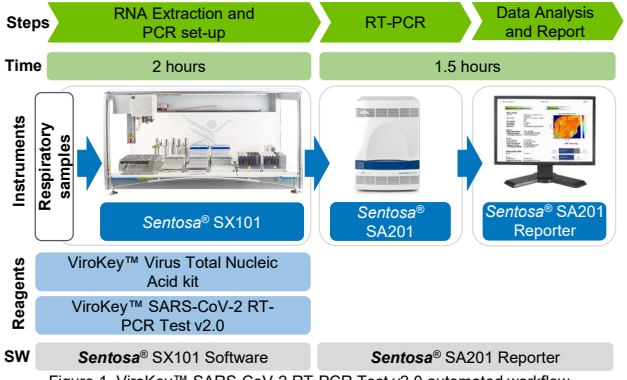

Figure 1. ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 automated workflow.

3. LoD at 9.2 GE/reaction

The LoD of ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 is determined using heat-inactivated SAR-CoV-2 virus and confirmed using 20 replicates samples which achieved at least 95% detection rate.

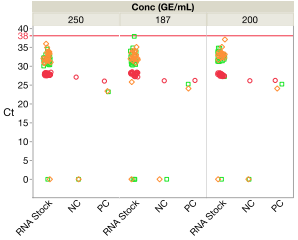

Table 1. Detection of LoD samples.

Conc (GE/mL)	Detection %		
	Orf1a	N	Extraction Control
250	100 (20/20)	100 (20/20)	100 (20/20)
200	95 (19/20)	95 (19/20)	100 (20/20)
187	97.1 (34/35)	97.1 (34/35)	100 (20/20)

Figure 2. Detection of samples at different concentrations using ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

4. No cross-reactivity with other microorganism

Table 2. Microorganism wet-lab tested for cross-reactivity.

Human coronavirus 229E	Influenza B virus, B/Lee/40
Human coronavirus OC43	Enterovirus, H
Human coronavirus HKU1	Human Respiratory syncytial virus, 18537
Middle East Respiratory Syndrome (MERS)	Rhinovirus 57, Ch47
Human coronavirus NL63	Haemophilus influenzae
Human adenovirus 1, Adenoid 71	Mycobacterium tuberculosis, H37Ra
Human parainfluenza virus 2, Greer	Streptococcus pneumoniae
Human parainfluenza virus 3, C243	Streptococcus pyogenes Rosenbach
Human parainfluenza virus 4a	Bordetella pertussis
Human parainfluenza virus 4b, CH 19503	Pooled human nasal wash
Influenza A virus (H3N2), A/Aichi/2/68	Mycoplasma pneumoniae
Influenza A virus (H1N1),strain A/Swine/Iowa/15/30	Legionella pneumophila

A total of 76 microorganisms tested for cross-reactivity with *in silico* analysis shows no cross-reactivity.

5. High specificity and zero interference

100% of **152,890** (as of 15th October 2020) SARS-CoV-2 genomic sequences found in GISAID and NCBI database are detectable by both Orf1a and N-gene primers and probes based

Table 3. Interference substance tested and its concentration.

Substance	Active Ingredient/s	Conc.
Nasal Wash (Flo®)	Sodium chloride, potassium chloride, calcium lactate pentahydrate	15% (v/v)
Nasal Spray/drops (Nazolin®)	Oxymetazoline HCl	15% (v/v)
Nasal corticosteroids	Fluticasone	5% (v/v)
Systemic antibacterial	Tobramycin	4 µg/mL
Antiviral drugs	Oseltamivir	3.3 mg/mL
Homeopathic relief (Prospan®)	Extract from ivy leaf (Hedera helix L. leaf), Potassium sorbate, anhydrous citric acid, xanthan gum, cherry flavour, crystallizing sorbitol syrup	5% (v/v)
Antimicrobial/antiviral/anesthetic lozenges (Dorithricin®)	Benzalkonium, Benzocaine, Tyrothricin	15% (w/v)
Whole blood	N.A.	2% (v/v)
Mucin		60 µg/mL
Pooled human nasal		N.A.

6. Clinical Sensitivity and Specificity

A total of 34 positive and 34 negative patient samples were tested against a comparator and ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

Table 4. Clinical Sensitivity and Specificity.

	Detection %	95% CI, %
Sensitivity	97.1 (33/34)	85.1, 99.5
Specificity	100 (34/34)	89.8, 100
PPV	100 (33/33)	89.6, 100
NPV	97.1 (34/35)	85.5, 99.5

Conclusion

We have developed a highly specific and sensitive SARS-CoV-2 nucleic acid test assay, ViroKey™ SARS-CoV-2 RT-PCR Test v2.0, which can contribute significantly to the diagnosis of COVID-19 patients. The availability of the automated workflow also allows minimal hands-on and high-throughput screening of patients for use in such a outbreak. On 22nd September 2020, ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 has been EUA-approved.