PIPETMAX® for Automation of RTqPCR Detection of SARS-CoV-2

CORONAVIRUS AND COVID-19

"Coronaviruses are enveloped, positive-sense single stranded RNA viruses belonging to the family Coronaviridae and the order Nidovirales and are broadly distributed in humans and other mammals (Huang, et al.,2020)." They are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry. The genomesize of coronaviruses ranges from approximately 26 to 32 kilobases, the largest for an RNA virus.

In humans, the viruses cause respiratory infections, including the common cold, which are typically mild, though rarer forms such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) can be lethal. In December 2019, a novel pneumonia outbreak was reported in Wuhan, China (Read, Bridgen, Cummings, Ho, & Jewell,2020) and was later identified to be caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Figure 1). This disease was officially named COVID-19 by the World Health Organization (WHO). Common symptoms of the infection include: respiratory symptoms, fever, cough, shortness of breath, and breathing difficulties. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death.

RT-qPCR FOR SARS-COV-2 TESTING

Reverse transcription qPCR (RT-qPCR) combines reverse transcription and qPCR, which is routinely used to detect causative virions from respiratory secretions.

Respiratory samples from humans are obtained and tested as described in the following steps (Figure 2).
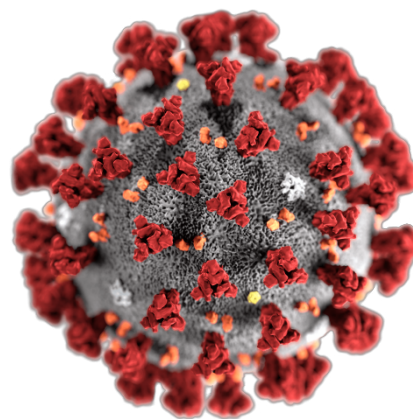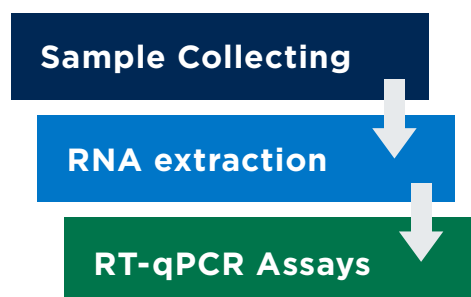

Figure 1
Rendering of a SARS-CoV-2 Virion

Figure 2
RT-qPCR Workflow of COVID-19

PIPETMAX® and RT-qPCR Automation

Gilson's PIPETMAX automated liquid handler removes the risk of manual error in sample transfers. The liquid handler has a volume range from 1 μL to 1200 μL and the ability to use 1 to 8 pipetting tips at any one time (Figure 3). The removable tray can also fit both 96- and 384-well plates. Dual pipetting heads automatically pick the required volume range when running, without changing the heads. With more than one removable tray, researchers can prepare the next experiment while the system is running (Figure 4).

Figure 4
PIPETMAX® Removable Tray

Figure 3
PIPETMAX® with TRILUTION® micro

RT-qPCR Workflow*

Nucleic Acid Extraction

Performance of RT-qPCR amplification-based assays depend on the amount and quality of sample template RNA. Viral RNA extraction procedures should be qualified and validated for recovery, purity, and specificity before testing specimens.

Commercially available extraction procedures have been shown to generate highly purified viral RNA when following manufacturer's recommended procedures for sample extraction.

Quality Control

Due to the sensitivity of RT-qPCR, these assays should be conducted using strict quality control and quality assurance procedures. It's best practice to use aerosol barrier (filter) pipette tips only and assay controls should be run concurrently with all test samples.

*The RT-qPCR protocol refers to guidance from the US CDC onFebruary 4th, 2020 and may differ in practice as the situationchanges. This article describes the use of PIPETMAX for setup ofRT-qPCR plates prior to thermocycling.
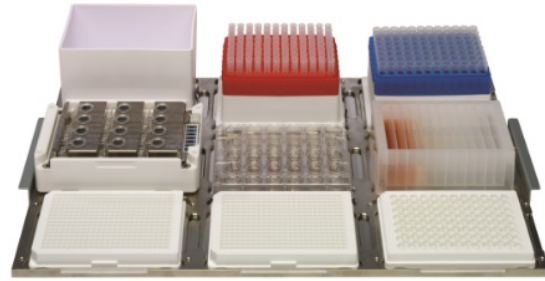
PIPETMAX Benefits

PIPETMAX is an ideal solution for the efficient processing of assays. Its small footprint easily fits into any lab. With an appropriate external UV/HEPA hood, it is the ideal tool for qPCR reaction preparation. Additionally, PIPETMAX helps researchers improve the accuracy, reproducibility, and consistency among the viral nucleic acid being processed. PIPETMAX also:

• Helps eliminate user-to-user variability

• Protects researchers from pathogens

• Is built using PIPETMAN® technology for transferring liquids with high accuracy and precision

• Is compatible with PIPETMAN® DIAMOND Filter Tips (Figure 5) to offer protection against aerosol contamination

• Offers an open system that's compatible with many reagents, kits, or protocols

Figure 5
Gilson BLISTER Filter Tips

OTHER AUTOMATED APPLICATIONS

qPCR methods, particularly those utilizing probes and equivalent fluorescent probe hybridization, have been widely adopted in the detection of various pathogens.

Automation of viral nucleic acid extraction and detection has been reported for enteroviruses, hepatitis B, hepatitis C, Epstein-Barr virus, herpes simplex virus, human papillomavirus, influenza B and SARS virus.[3]

SUMMARY

Gilson liquid handling products, such as PIPETMAX, can be implemented into the RT-qPCR workflow for detection of SARS-CoV-2.

Growing testing demands, expanding assay menus, and a need for increased productivity while ensuring cost-savings and quality resulted in the development of more automated, integrated systems with minimal user interactions in molecular biology laboratories. Gilson automated liquid handling products are implemented in various aspects of the sample workflow, including pre-analytical sample handling, nucleic acid extraction, and PCR plate setup with varying degrees of automation and flexibility, and to improve process efficiency and standardization.

PIPETMAX BUNDLE

PRODUCT DESCRIPTION
PIPETMAX 268 W STANDARD COVER
PIPETMAX MAX8X200 PIPETTE HEAD × 1
PIPETMAX MAX8X20 PIPETTE HEAD × 1
PIPETMAX 268 TRAY 384 WELL × 1
MICROAMP SHORT 96 PCR TUBE RACK × 6
PIPETMAX TIP RELOAD BLOCK × 5
RACK,CODE 496 PCR TUBES ×1
BIN, TIP DISPOSAL ANNEALED ×1
RACK,CODE 424 PT PSV TMP FLP CAP 1.5-2ML × 1
RACK,CODE 496 PT PSV TMP 96 PCR TUBE × 1
DSF200ST BLISTER REFILL 384-Certified (960 tips/pack) × 3
DSF10ST BLISTER REFILL 384-Certified (960 tips/pack) × 3
Software, TRILUTION micro v3 for PC ×1
Computer for PIPETMAX ×1
Installation and Training- 1 Day ×2

PIPETMAX Specifications

MAXIMUM PERMISSIBLE ERRORS						
	Pipette Head	(µL)	Error (µL)	Error (µL)	Error (%)	Error (%)
For Transfers	MAX 8×20	1	±0.08	≤0.05	8.00	5.00
		10	±0.15	≤0.10	1.50	1.00
		20	±0.25	≤0.12	1.25	0.60
	MAX 8×200	20	±0.50	≤0.16	2.50	0.80
		100	±1.00	≤0.30	1.00	0.30
		200	±2.00	≤0.50	1.00	0.25
	MAX 4×1200	50	±4.00	≤0.70	8.00	1.40
		120	±4.00	≤0.70	3.33	0.58
		600	±6.00	≤1.50	1.00	0.25
		1200	±9.60	≤1.80	0.80	0.15

	Pipette Head	PIPETMAN® DIAMOND Tips	PIPETMAN® Pipette Head Volume Range (µL)
PIPETMAN® DIAMOND Tips (Sterilized option available for some tips)	MAX 8×20	DL10	1-10
		DF10	1-10
		DF30	1-20
		D200	1-20
	MAX 8×200	D200	20-200
		DF200	20-200
	MAX 4×1200	D1200	100-1200
		DF1200	100-1200

REFERENCES

1. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y.,... Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan,China. The Lancet, 395(10223), 497–506. doi:10.1016/s0140-6736(20)30183-52.

2. Read, J. M., Bridgen, J. R., Cummings, D. A., Ho,A., & Jewell, C. P. (2020). Novel coronavirus 2019-nCoV: early estimation of epidemiological parameters and epidemic predictions. doi:10.1101/2020.01.23.200185493.

3. Tewari, D., Zellers, C., Acland, H., & Pedersen, J. C.(2007). Automated extraction of avian influenzavirus for rapid detection using real-time RT-PCR.Journal of Clinical Virology, 40(2), 142–145. doi:10.1016/j.jcv.2007.07.011