End-to-end workflow solutions for low- to high-throughput SARS-CoV-2 testing

Introduction

The novel coronavirus strain that was first identified in Wuhan, China, in December 2019 spread rapidly throughout the world, and has been named SARS-CoV-2.

Robust tests that can be mass-produced at several million per week and routinely used in research laboratories globally are critical to understanding how to control the spread of the virus and eventually eradicate it. Currently there are three different research-based testing options for SARS-CoV-2: detection of virus-specific antibodies in the blood, detection of viral proteins (antigens), and detection of viral nucleic acid by PCR. Antigen and PCR tests determine whether a sample is currently infected with the coronavirus, while an antibody test can determine if the subject was infected more than 2–4 weeks prior.

Quantitative reverse transcription PCR (qRT-PCR), which detects and measures levels of RNA, is the most sensitive, specific, and reliable method for detection of SARS-CoV-2. Since SARS-CoV-2 is an RNA virus, its genome is first converted to DNA by reverse transcription, and then PCR is performed to amplify and determine the levels at which the pathogen is present. This workflow requires a range of materials. Thermo Fisher Scientific offers a robust portfolio of reagents and instruments that are currently used in research laboratories globally.

In order to stay one step ahead of the virus, some labs will need to quickly scale up to processing thousands of research samples per day, optimize workflows, and select the correct products for use in high-throughput settings. This white paper describes workflow options for qRT-PCR–based SARS-CoV-2 research, highlighting challenges, solutions, and scenarios for scaling up processing from a few samples to thousands of samples per day. Emphasis is on the viral RNA purification process, which is a critical first step of the qPCR-based assay for SARS-CoV-2.

SARS-CoV-2 research-based testing options

Laboratories need to decide which SARS-CoV-2 qPCR-based testing workflow is right for them, depending on their goals, number of samples to be processed daily, personnel, lab equipment, and other factors.

A laboratory can use any RNA purification kit, qRT-PCR test, and instruments to develop a unique workflow. For instance, the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit can be used with any downstream qRT-PCR test. This option allows labs the flexibility to design a process that works for them. However, it also requires significant process optimization and test verification, which can be time-consuming.

The workflow starts with research samples being shipped to the laboratory where they are accessioned and transferred into 96-well plates. Next, viral RNA is extracted, qRT-PCR is performed, and data analyzed (Figure 1).

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Figure 1. qPCR-based detection of RNA from SARS-CoV-2.

Research sample collection, accessioning, and transfer into 96-well plates

Standard sample collection kits for SARS-CoV-2 testing use nasopharyngeal swabs and tubes containing either universal transport medium (UTM) or viral transport medium (VTM). Other research sample types include oropharyngeal, nasal, and mid-turbinate swabs, nasopharyngeal aspirate, bronchoalveolar lavage (BAL), sputum, or saliva. Tubes with UTM and VTM maintain the activity of the virus and stabilize it during transport, while preventing growth of bacteria and fungi. Some laboratories follow protocols for virus inactivation before RNA isolation, while others proceed with RNA isolation and rely on the lysis buffer in the first step of purification process to inactivate the virus. It is crucial that the technician be aware of the status of inactivation to avoid virus spread.

During sample acquisition, laboratories receive a tube containing the research sample in a biohazard bag with bar codes and matching information. The sample tube is cleaned (typically with 70% ethanol), bar-coded, and either processed further manually or placed in an automated instrument to be scanned and arrayed into a bar-coded 96 deep-well plate, which will then be used for RNA purification.

For many labs, up-front sample accessioning and handling is a major bottleneck in the SARS-CoV-2 research workflow, as it is largely done manually. Lab personnel must carefully track and transfer individual samples into intermediate tubes in a laborious process of de-capping and capping, and then dispense part of the volume into the 96-well plates so that samples are ready for the RNA purification and subsequent PCR. Additional complications can arise from diverse incoming samples types, tubes, and volumes. The process can be only partially automated and requires liquid handling robots, which may not be readily available to all laboratories.

A manual tube de-capping and capping operation takes ~10 min for 96 samples.

Solutions include a manual torquer at ~5 min per 96 samples
Or
A hands-off liquid handler at ~15 min per 96 samples.

Samples must next be transferred from the tube to a 96 deep-well plate.

Manual operation with a single-channel pipette takes up to 1 hour for 96 samples, taking into account that documentation needs to be filled out and all manipulations are performed in a BSL-2 hood.

By contrast, using a liquid handler takes ~5 min for 96 samples.

Overall the up-front sample processing typically takes 1–2 hours for 96 samples.

Many labs rely on a laboratory information management system (LIMS) customized to their processes and operational needs to automate workflows, integrate instruments, and manage sample data.

Viral nucleic acid isolation

SARS-CoV-2 RNA can be purified quickly and efficiently from samples using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit or the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. Both products are magnetic bead–based, which enables high-throughput sample processing using either magnetic stands or specialized purification instruments. The kits contain the same reagents and perform similarly; the only difference is the source material for the beads.

Features of the MagMAX Viral/Pathogen Nucleic Acid Isolation Kits include:

- Automation-ready protocols designed for Thermo Scientific™ KingFisher™ Flex, Duo Prime, and Presto instruments
- Fast procedure allows for 96 samples to be processed in 1 hr
- Flexible protocol accommodates sample volume inputs of 200–400 µL of various transport media and body fluids
- No need for carrier RNA
- Elution volumes ranging from 50 µL to 100 µL

Both MagMAX Viral/Pathogen kits can be automated with the KingFisher Flex system for fast, easy, and effective high-throughput nucleic acid isolation. The KingFisher Flex instrument is a versatile benchtop instrument for processing up to 96 samples per run. Features of this system include:

- High-throughput and flexible purification of 1–96 samples per run
- Easy to install and operate (set up and ready to run in 10 min or less)
- Choose from two plate formats for wide volume range (20–5,000 µL)
- Download existing protocols from a library, or easily customize protocols using Thermo Scientific™ BindIt™ Software

For SARS-CoV-2 pathogen detection, there are several workflow options, from manual to fully automated, depending primarily on the desired throughput (i.e., number of samples processed per day) (Table 1).

Table 1. Solutions for low-, medium-, and high-throughput SARS-CoV-2 testing.

Workflow	RNA purification plate setup	Viral RNA purification method	Plastics used per run	Number of personnel	RNA purification time (total and hands-on)	Runs per instrument per day	Number of samples processed in 8 hr (for 1 instrument)
Manual Magnetic stand	Multichannel pipette (8 or 12 channels)	Manual magnetic stand	96 deep-well plate and elution plate	4–6	~1–2 hr All hands-on time	4–5	Up to 500
Semi-automated KingFisher Flex instrument	Multichannel pipette (8 or 12 channels)	Semi-automated KingFisher Flex instrument	4* KingFisher 96 deep-well plates 96 tip comb Tip comb plate	3–5	~1 hr ~50% hands-on time	8	700–1,000
Semi-automated KingFisher Flex instrument	Multidrop plate filler and pipette	Semi-automated KingFisher Flex instrument	4* KingFisher 96 deep-well plates 96 tip comb Tip comb plate	3–5	~1 hr ~40% hands-on time	8	700–1,000
Mostly automated Liquid handler/ KingFisher Presto instrument	Automated liquid handler	Automated KingFisher Presto instrument integrated with liquid handler	4* KingFisher 96 deep-well plates 96 tip comb Tip comb plate	2–4	~1 hr ~20% hands-on time	8	700–1,000
Fully automated Liquid handling robot	Liquid handling robot	Liquid handling robot	System-dependent	2–3	System-dependent (1–4 hr) ~10% hands-on time	System-dependent, ~8	System-dependent, 1,000+

* The 2-wash protocol uses 4 plates: sample plate, wash 1, wash 2, and elution plate. The 3-wash protocol uses 5 plates: sample plate, wash 1, wash 2, wash 3, and elution plate. Either protocol can be used with the MagMAX Viral/Pathogen kits.

Manual

Magnetic beads using magnetic stand

This method involves manually filling the 96-well plates with MagMAX kit reagents, adding the samples, and completing all the extraction steps on the magnetic stand using a multichannel pipette (8- or 12-channel). Note that the entire 1–2 hour protocol time is hands-on for this approach. However, this option allows processing of a reasonable number of samples per day, using only one 96 deep-well plate for RNA isolation and one elution plate. It is ideal for low-throughput labs, many of which process fewer than 200 samples per day, or medium-throughput labs, which routinely process several full plates per day on sample purification instruments, but occasionally process a few samples manually using partially filled plates.

Semi-automated

KingFisher Flex instrument and magnetic beads

This is one of the most widely used workflows for medium-throughput SARS-CoV-2 testing. Users manually fill five 96-well plates with MagMAX reagents using a multichannel pipette. Then samples are transferred. Next, all the plates and the tip comb are loaded onto the deck of a KingFisher Flex instrument. The viral RNA isolation protocol is initiated, which takes only 25 minutes. Hands-on time is dramatically reduced compared to the manual approach. A typical lab using 1–4 KingFisher Flex instruments can easily process 1,000–4,000 samples per day.

Semi-automated

KingFisher Flex instrument and Thermo Scientific™ Multidrop™ Combi Reagent Dispenser

This option is identical to the previous one, except plates are filled with MagMAX kit reagents using a Multidrop reagent dispenser, instead of a multichannel pipette. The Multidrop instrument handles a wide selection of plates and volume ranges and provides fast dispensing and high-throughput operation (Figure 2).

The Multidrop reagent dispenser can fill 96-well plates with reagents for the entire day. The binding bead mix should be plated on the day of preparation; however, wash and elution buffers can be plated 2–3 days in advance (plates can be sealed and stored at room temperature). Besides the sample and MS2 phage control, the only other component that needs to be added manually with a multichannel pipette is Invitrogen™ Proteinase K.

Features of the Multidrop reagent dispenser include:

- Precise dispensing over a 0.5–2,500 µL range, ensuring reproducible assay data
- Accommodates microplates with 6–1,536 wells and plate heights of 5–50 mm
- Visual icon-based graphic display makes it easy to use and program, even without training
- Minimal dead volume and back-flushing reduces reagent costs
- Easy-to-use Thermo Scientific™ FILLit™ Software provides increased flexibility and functionality
- Full robotic compatibility enables increased throughput
- Uses 8-channel detachable and autoclavable dispensing cassettes that are standard across the Multidrop range

Mostly automated

KingFisher Presto instrument with liquid handler

This workflow can be used to process 1,000 samples per day with fewer personnel. A liquid handler from Hamilton or Tecan is combined with a KingFisher Presto instrument for a mostly automated protocol.

KingFisher Presto instrument features:

- Automated purification of 1–24 or 1–96 samples at a time
- Volume range of 50–5,000 µL
- Minimized size for easy fitting to automation systems
- Heating capability for applications requiring elevated temperatures
- Designed to integrate with several liquid handling instruments

A typical setup using a liquid handler with a KingFisher Presto instrument takes about 20 minutes, and the run itself about 40 minutes. Liquid handlers dramatically accelerate plate filling and sample handling and are a must-have for high-throughput testing (see Figure 2).

Workflows utilizing:

	Multichannel pipette	Multidrop reagent dispenser	8-channel liquid handler	96-channel liquid handler
Time per set of plates (3 wash, 1 elution) for 96 samples	~3 min	~2 min	~5 min	~2 min

Figure 2. Options for filling 96-well plates with reagents for purification of viral RNA, ranging from manual to fully automated. Note that for multichannel pipettes all of the time is hands-on, in contrast to the Multidrop reagent dispenser and liquid handlers, which can fill hundreds of plates per day with minimal up-front manual setup.

Fully automated

Liquid handling robots

This integrated purification solution allows processing thousands of samples per day with minimal personnel. It involves liquid handling robots; with minimal setup time, the run typically takes 1–4 hours for different systems.

To summarize, there are several options for low-, medium-, and high-throughput purification of viral RNA. With appropriate space and infrastructure, labs can scale up from 10 to thousands of samples per day by gradually increasing the number of KingFisher Flex instruments, liquid handlers, or other instruments. However, it is important to note that all options will require lab personnel, as none of these setups is a complete "walk-away" solution. The exact number of people will depend on several key variables: the number of samples to be processed per day, number of shifts, and type and number of instruments in the lab (KingFisher instruments, liquid handlers, qPCR, etc.).

It is beneficial to have dedicated teams focused on different parts of the workflow. For example, in a segregated workflow for the semi-automated approach, one specialist (or one team) would be dedicated to documentation, sample accessioning, and transfer. Another specialist (or team) would be dedicated to viral RNA isolation. Yet another team would be focused on running the qRT-PCR assays, data analysis, and report generation. This approach accelerates the process, minimizes opportunities for error, and distributes manpower. Note that while instruments are running, the next set of plates can be prepared as overlap to increase daily throughput.

qRT-PCR and data analysis

Once viral RNA is extracted from samples, the next step is the qRT-PCR test, which determines the levels of the viral RNA and generates a primary analysis. qRT-PCR can be performed using Applied Biosystems™ TaqMan® Assays on qPCR research instruments such as the Applied Biosystems™ 7500 Series or QuantStudio systems. The software calculates and analyzes C_t values for the controls and specimens of each run.

In general, the entire SARS-CoV-2 testing workflow takes 3–4 hours, from receiving the sample to completion of data analysis.

Summary

There are several workflow options for qRT-PCR–based SARS-CoV-2 testing, and each laboratory determines their process based on their unique goals, needs, constraints, and tradeoffs.

For low- and medium-throughput applications, the optimal workflow would use a KingFisher Flex instrument with a Multidrop reagent dispenser, and an Applied Biosystems real-time PCR instrument.

If scale-up to high throughput is desired, laboratories can use up to 16 combinations of KingFisher purification systems and Applied Biosystems real-time PCR systems with two Multidrop reagent dispensers.

Laboratories can also use a liquid handler, KingFisher Presto instrument, and Multidrop reagent dispenser to increase the throughput of sample extractions, combined with an Applied Biosystems real-time PCR system for downstream analysis.

With more resources and a goal to focus on high-throughput sample processing, labs can invest in liquid handling robots (instead of KingFisher instruments) and the Applied Biosystems™ QuantStudio™ 7 Flex instrument (instead of the 7500 Fast Real-Time PCR System) to process thousands of samples per day.

Importantly, the handling of a sample, accessioning, and transfer into 96-well plates for downstream RNA purification remains the major bottleneck in SARS-CoV-2 testing workflows. Viral RNA purification and qPCR analysis can be performed quickly, and without any major hurdles.

With a comprehensive portfolio of products, Thermo Fisher Scientific is your trusted partner for pathogen detection, as well as epidemiological surveillance, vaccine development, and the laboratory equipment needed to advance your research.

Ordering information

Product	Quantity	Cat. No.
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	5400630
KingFisher Flex Purification System with 96 KF Head	1 system	5400620
KingFisher Presto Purification System with 96 DW Head	1 system	5400830
KingFisher Duo Prime Purification System	1 system	5400110
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	Up to 200 rxn	A42352
	Up to 2,000 rxn	A48310
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	Up to 2,000 rxn	A48383R
DynaMag-96 Side Magnet	1	12331D
DynaMag™-96 Bottom Magnet	1	12332D
M4RT MicroTest Tubes	72 tubes	R12505
M4RT MicroTest Flocked Swab Kits	100 kits	R12552
M4RT Kit w/ Micro-Tipped Flocked Swab	100 kits	R12566
M4RT Universal Swab Kit	100 kits	R12578
7500 Fast Real-Time PCR System, laptop	1 system	4351106
QuantStudio 5 Real-Time PCR System, 96-well, 0.1 mL	1 system	A28138
QuantStudio 5 Real-Time PCR System, 384-well	1 system	A28140
QuantStudio 6 Pro Real-Time PCR System, 96-well, 0.2 mL	1 system	A43159
QuantStudio 7 Pro Real-Time PCR System, 96-well, 0.2 mL	1 system	A43162
QuantStudio 7 Flex Real-Time PCR System, 384-well, desktop	1 system	4485701
QuantStudio 12K Flex Real-Time PCR System, OpenArray block, AccuFill System	1 system	4471090

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