Application note

A high-throughput PCR testing workflow for molecular diagnostics

Enabling large-scale COVID-19 testing through automation

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Keywords

COVID-19; high-throughput testing; PCR data analysis; lab automation; artificial intelligence; sample-to-result; performance optimization; analysis software; qPCR; RT-PCR; RT-qPCR; SARS-CoV-2 detection and screening

Introduction

Large-scale SARS-CoV-2 testing initiatives that have been set up across the world have underscored the need for a high degree of automation and dependable supply chains across the sample-to-result workflow. Because of unprecedented volumes in both screening and diagnostics, efficiencies across sample accessioning and preparation, extraction, PCR cycling, data analysis and reporting have become instrumental in providing the required throughput capacity as well as maintaining accuracy and result reliability required for clinical diagnostics.

As respiratory illness seasons come and go, the need for broader panels to screen for COVID-19 and other respiratory infections like influenza will increasingly pressure labs to have set-ups that can adapt to multiplexing while maintaining a throughput that keeps up with demand. In this application note, we discuss the high-throughput capability of LGC, Biosearch Technologies' SARS-CoV-2 Real-Time and End-Point RT-PCR Test, the EUA request submitted to the US FDA, and the advantages of the automation that is further enabled by including UgenTec's FastFinder software into the testing workflow.

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Workflow overview
An integrated workflow, from sample to result

This workflow integrates with a laboratory's existing laboratory information management system (LIMS) solution, connects with instrumentation, and automates the entire workflow — from sample preparation and extraction to PCR amplification and detection through data analysis and report generation. Figure 1 illustrates the end-to-end workflow a sample will traverse from its accessioning to reporting the clinical result.

Systems integration	UgenTec's FastFinder software integrates into existing LIMS and IT infrastructure: ● Connects to front end of liquid handling or sample prep ● Collects sample ID for end-to-end tracking ● Syncs into LIMS for downstream reporting.
Nucleic acid preparation	Samples are prepared into plates and extracted by the oKtopure™ automated nucleic acid extraction system using sbeadex™ viral RNA purification kits. Easily track samples down to each well per plate at any time with the FastFinder Workflow and view status in real-time, across all instruments.
Amplification and detection	PCR assays designed with Biosearch Technologies' reagents are loaded into the IntelliQube™ system, which support both qPCR (up to 4,500 tests per day) and end-point PCR (up to 35,000 tests per day) assays. Results are automatically transferred to the FastFinder Analysis, where artificial intelligence (AI) algorithms ensure optimal curve or end-point calling.
Data analysis	Data analysis is fully automated from raw cycler data to called result, so only exceptions are presented to the qualified clinical laboratorian for final calling resolution. FastFinder Analysis automates the assay decision logic to generate the sample result. It eliminates the manual steps in analysis and result calling, such as combining multiple markers, assessing positive and negative controls, validating QC, evaluating replicates, checking thresholds, and flagging exceptions.
Report generation	Automated result generation, digital archiving, audit trails for samples across the entire workflow.
Lab intelligence	Visual dashboards track positive rates, Quality Control (QC) and lab key performance indicators (KPIs) across instruments and sites for real-time access.

Table 1. Sample-to-result for real-time qPCR and end-point PCR testing for SARS-CoV-2.

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Accurately call, certify and return results for high-throughput, mass testing

Workflow

Workflow software enables true sample-to-results, reducing manual steps and errors.

Analysis

Artificial intelligence for real-time and end-point PCR data analytics. Automate assay, speed up turnaround time and reduce errors.

Insights

Get operational insights across samples, assays, instruments and labs. Track lab performance and visualize QC.

Reception | Sample prep | RNA isolation | Liquid handling | PCR | Analysis and curve calling | Interpretation | Reporting and approval
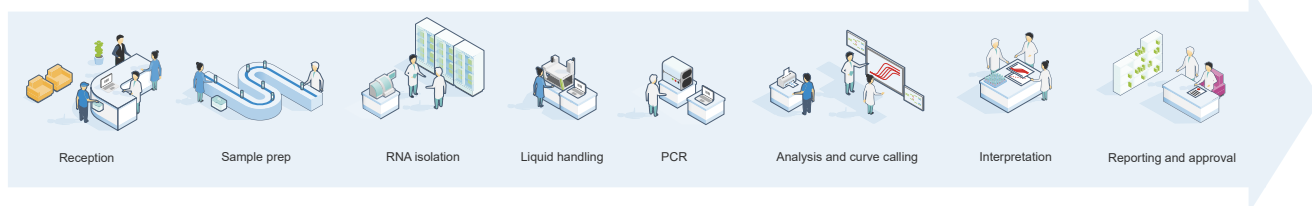
Figure 1. Testing workflow from sample acquisition to test results for high-throughput, mass testing. Molecular testing labs require robust, scalable analytics to provide timely, accurate answers and confidence in the sample results. At massive scale, reviewing the results manually is no longer an option, especially when the expert lab staff is already strained. Because each test collects multiple data points, a scalable and robust data analysis pipeline becomes an instrumental component to enable scalability, reduce turnaround times and increase confidence in results.

Automation redefines lab efficiency and confidence in results

A PCR sample-to-result workflow for high-throughput testing (Figure 1) requires automation optimized across every step for maximum reliability and efficiency. Thousands of test results means there are thousands of data points to assess, requiring a robust informatics set-up that can analyze, review, interpret and report at the same pace.

This requires the informatics to link across instruments and connect back to LIMS to introduce novel efficiencies to the lab. These include the capacities to:

- **Eliminate manual interactions to remove human error and manipulation.** Manual transfer of results, queries and lookup, report generation, upload/download of files and data entry into LIMS systems are all opportunities for bottlenecks, errors and delays.

- **Trigger expert review only when warranted.** As the bulk of clear negatives and positives are assessed automatically by FastFinder AI, *only exceptions or ambiguous results trigger human expert review* to optimize the effort of lab personnel.

- **Access the status of samples at any given time.** Keep track of samples across all plates and instruments with full traceability for any operators handling them. Troubleshoot and manage exceptions and ambiguous results by tracking any sample to the specific multiplex well in a matter of seconds.

- **Maximize operational efficiency in the lab.** Provide tools for worklist and workflow monitoring, dashboarding key QC metrics, and visual dashboarding on key metrics such as positive rates, sample throughput and turnaround times.

- **Provide a "birds-eye operational view".** Support real-time visual overviews of lab KPIs across all instruments and even sites.

In this application note, we examine how high-throughput PCR result calling and analysis is enabled by AI and automation. We also demonstrate how UgenTec's FastFinder software connects with Biosearch Technologies' IntelliQube integrated PCR and qPCR system to create an optimized workflow that yields traceable and scalable data analytics and reporting.

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We focus on three key efficiencies: 1) instrument integration and IT connectivity, 2) data analysis and interpretation and 3) a ready-to-deploy, high-throughput configurable system.

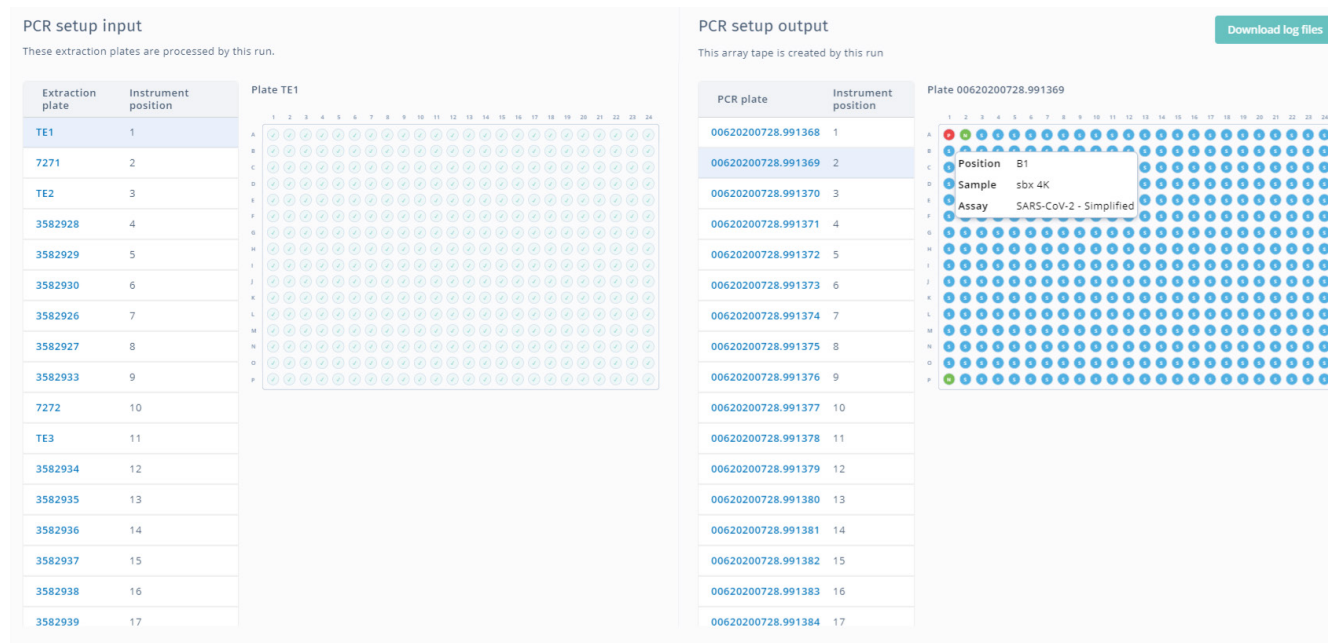### Efficiency 1: Instrument integration and IT connectivity

Figure 2. FastFinder gives users control of the sample workflow, connecting directly to instruments and reading sample well location and metadata details for seamless full sample tracking. This screen illustrates one stage in the workflow where extraction plates are transferred to Array Tape™, providing interactive plate maps with informative detail annotations.

FastFinder seamlessly integrates with the IntelliQube system by automatically picking up sample identifiers and plate locations directly from the instrument, avoiding the introduction of errors caused by manual copy-paste actions or file uploads. From there, samples are fully tracked down to the well, across plates or on the IntelliQube system's unique Array Tape consumable. One of the workflow steps is shown in Figure 2, and clearly delineates the input and output of steps in the workflow, allowing the user to get detailed information on wells, samples, instruments, assays and more.

With clear, visual queues and sample worklists, users have full control and visibility of samples in the workflow – not only to confirm that the reaction went as expected, but also for troubleshooting: was a sample tested before? Was the source material sufficient? Was the extraction step leading into the PCR assay preparation successful? What touchpoints (instrument, plate, operator, others) did the sample encounter?

The FastFinder workflow enables users to track a sample along its complete journey through the lab – from liquid handler to end result.

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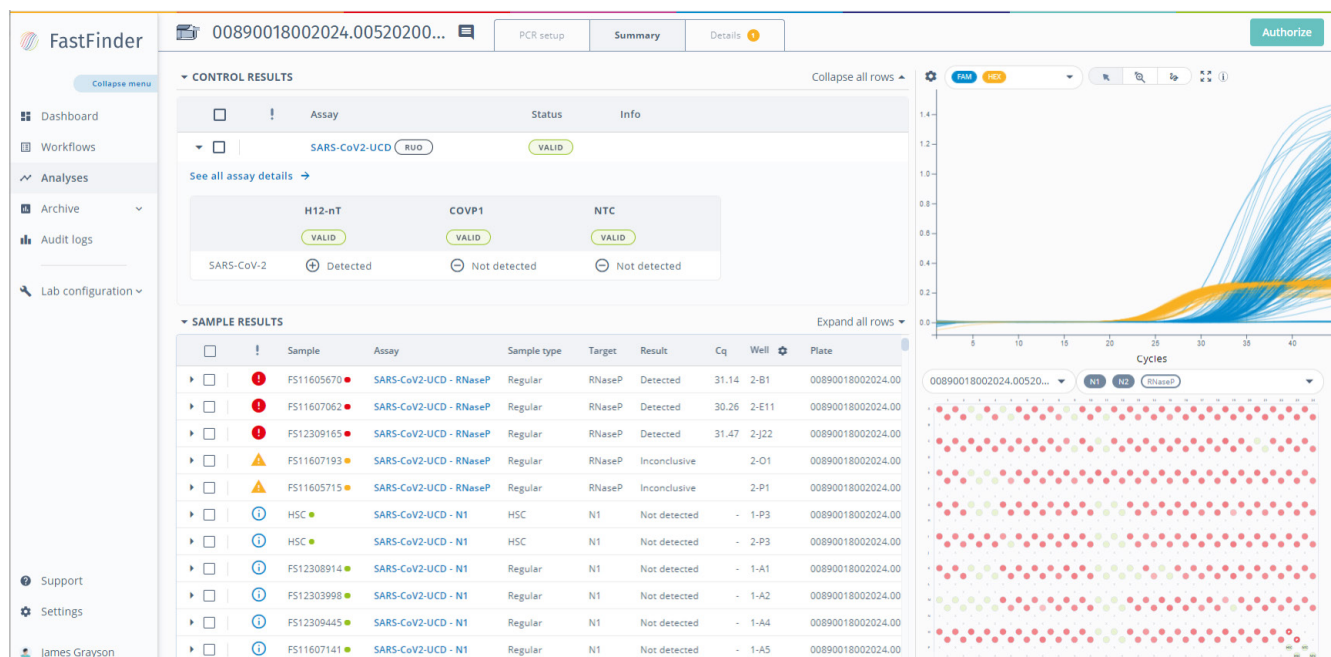
Efficiency 2: Data analysis and interpretation

Figure 3. FastFinder Analysis fully automates the data analysis from raw fluorescence data to called sample result. This summary screen shows a comprehensive view of the control system, an interactive curve viewer, flagged exceptions that need review, positive samples and an interactive plate viewer, all at a glance.

Specific needs in a high-throughput setting include not only automating the clearly negative and clearly positive samples, but also optimizing the exceptions that occur. With large numbers of samples, the aggregate workload on personnel to manually review and sign off becomes significant, time-consuming and unmanageable. It is therefore important to minimize this manual review time as much as possible and only require expert attention when it is necessary. To this end, we showcase the use of FastFinder's automated data analysis capabilities and workflow orchestration capabilities to go beyond typical instrumentation software

and optimize analytical performance to minimize manual review time.

Of note, full audit trails and sample chains of custody through the lab – across plates, instruments and analytics – is essential for QC, troubleshooting and traceability in a high-throughput setting. Figures 3 and 4 illustrate sample tracking through the workflow, with operators, plates, instruments and timestamps identified in a visual, user-friendly manner.

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Figure 4. This FastFinder screen shows the full chain of custody for a selected sample. With one click, a detailed view appears with a visual audit trail of the path a sample took through the lab. This snapshot includes identification of the lab technicians who handled the sample, barcode and timestamp information, and the instruments and plates where the sample was processed as well as the review and sign-off details.

Efficiency 3: Ready-to-deploy, high-throughput configurable system

Convenient, pre-configured workflows (Figure 5) enable fast deployment, minimize the time across installation, validation and go-live, incorporate data analysis and reporting features, and offer multiplexing capabilities. This configurable system offers capacities to:

- **Remove guesswork.** Eliminate any guesswork for workflows with the IntelliQube integrated PCR platform to automate PCR prep, amplification and detection for either qPCR and end-point PCR;

- **Implement immediately.** With all assay-specific aspects, including curve calling algorithm parameters, decision tree logic

to perform result calling and reporting rules, embedded in a pre-configured Assay Plugin, deploying a workflow with the FastFinder Analysis is immediate;

- **Adapt to needs.** Labs that need the flexibility to expand capabilities can add additional workflows, modify assays or create lab developed tests (LDTs) to fit specific needs, or run multiple assays (such as broader panels, or qPCR alongside end-point assays). The configurable platform enables labs to run additional assays, assess multiplexed targets and manage multiple workflows in parallel while maintaining full traceability.

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Figure 5. The FastFinder Workflow allows the lab to hit the ground running with pre-configured workflows, and to configure a custom workflow to ensure their needs are met when changes to the assays, instrumentation and formats are required.

Conclusion

To achieve mass testing, it is advantageous to leverage automation to eliminate manual steps–from sample extraction and preparation to data generation to analysis and interpretation that are crucial for executing high-throughput testing. The integration of UgenTec's FastFinder software with Biosearch Technologies' instrumentation reveals an optimized end-to-end workflow capable of large-scale COVID-19 testing on a single system. This workflow conveniently integrates seamlessly into the existing LIMS as a ready-to-deploy, scalable solution capable of massive input of patient samples while streamlining high-throughput data generation for faster results.

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Case study: UC Davis mobilizes to conduct mass COVID-19 testing

An example of a comprehensive workflow was demonstrated with the deployment of a high-throughput qPCR-based workflow for mass SARS-CoV-2 testing on the University of California, Davis campus and the City of Davis, California, USA. This collaborative effort involved development of a high-capacity collection strategy, combined with deployment of an integrated solution featuring Biosearch Technologies' IntelliQube instrumentation and UgenTec's FactFinder software for efficient data handling. The complete and efficient workflow was determined in less than two months and continues to evolve.

Challenge:	• *Testing frequency:* Provide free weekly testing coupled with increased frequency of more strategic testing to the entire city of Davis, California, USA (population 69,413)[1]. • *Limitations:* Existing platforms have limited throughput. Need to minimize the turnaround time and still accurately call, certify and return results.
Solution:	• Develop a high-capacity collection strategy. • Deploy the integrated Biosearch Technologies IntelliQube and UgenTec FastFinder solution for efficient data handling. • Allow for a throughput capability of ~3,000 samples per day.
Results:	• Established a sample-to-result workflow in less than two months while continuing to evolve with changing needs and demand. • As an example underscoring time efficiency, during a randomly selected shift, the workflow processed three 768-well assays per run with the IntelliQube instrumentation enabled by Array Tape consumable, for a set of 2,304 PCR reactions. With FastFinder, only five of these PCR curves were raised for human review.

[1]*According to estimates of the US Census Bureau: https://www.census.gov/quickfacts/daviscitycalifornia*

"The goal is to provide at least weekly testing combined with more frequent strategic testing. This **requires rapid data analysis** software that minimizes the time needed to accurately call, certify, and return results."

- Professor Richard W. Michelmore, PhD, University of California, Davis

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