Case Study: intelligent software for multiplex qPCR interpretation support and workflow automation in a routine diagnostic setting

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What you will learn

In this case study, you will gain insights into:

- What challenges exist when automating and validating the interpretation of a complex lab developed respiratory panel for routine diagnostic use
- How OLVZ adopted software automation to decrease hands-on interpretation time
- How robust QC tools allow you to track run quality across lots and instruments
- How automation cuts time across workflow components: interpretation, reporting, and downstream LIMS integration

> With FastFinder, we **cut our qPCR curve analysis and processing time by two** — while increasing our overall accuracy for complex, multiplex tests
>
> **- Karen Dierickx, Molecular Biologist at OLVZ Aalst**

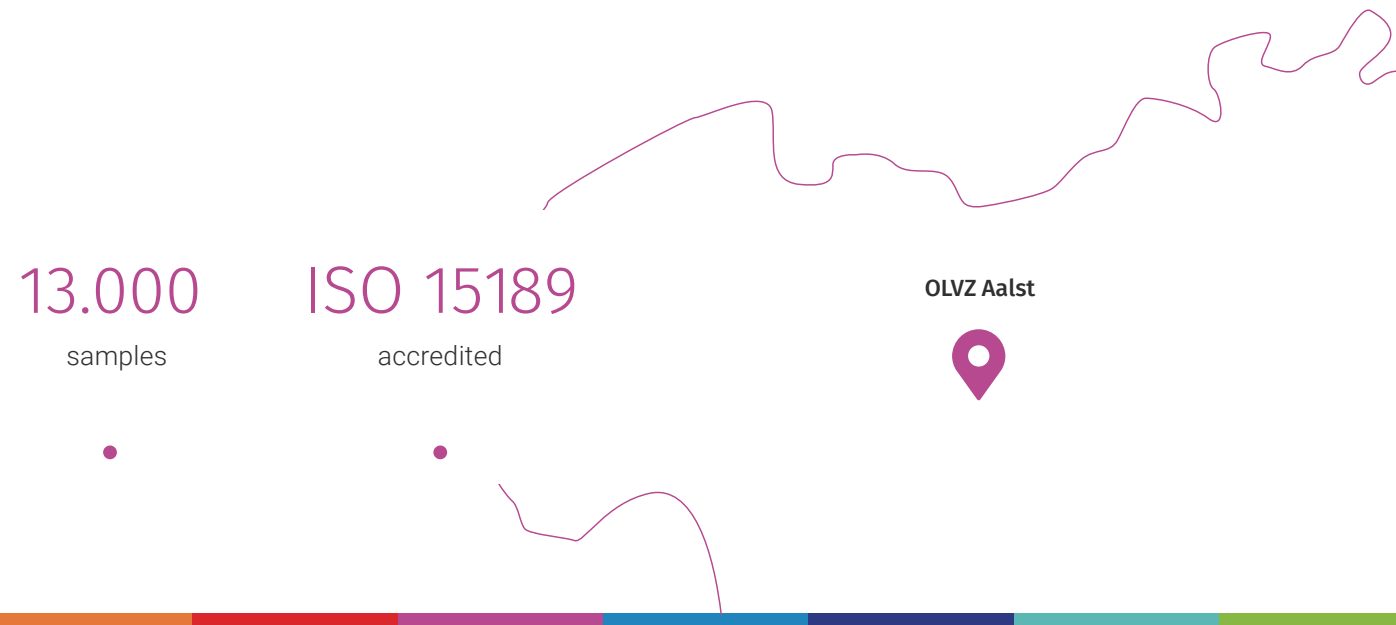
Introduction to the workflow at the OLVZ Aalst Hospital lab

» The molecular biology lab at OLVZ Aalst (www.olvz.be) is a diagnostic lab that runs about 13000 molecular samples per year, serving one of the largest regional non-academic hospitals in Flanders, Belgium.

The lab activities span across virology, bacteriology and hematology applications. Starting from patient samples, typical applications include testing for viral and bacterial infections, detection of acquired diseases, and identification of micro-organisms, supporting both initial diagnosis and follow-up. The molecular biology lab uses PCR, real-time PCR, sequencing, and genotyping assays.

> **OUR LAB ACTIVITIES SPAN ACROSS VIROLOGY, BACTERIOLOGY AND HEMATOLOGY APPLICATIONS.**

The lab is accredited and audited annually under ISO15189, an international standard that governs medical laboratory requirements for quality and competence. The lab adheres to strict quality control across assays, accommodation and administration. Furthermore, accreditation is mandatory in order to be amenable to reimbursement under the Belgian RIZIV governmental health payor system.



Workflow overview

» Currently, the workflow at the molecular biology lab runs in an automated fashion. The key steps are depicted in Figure 1.

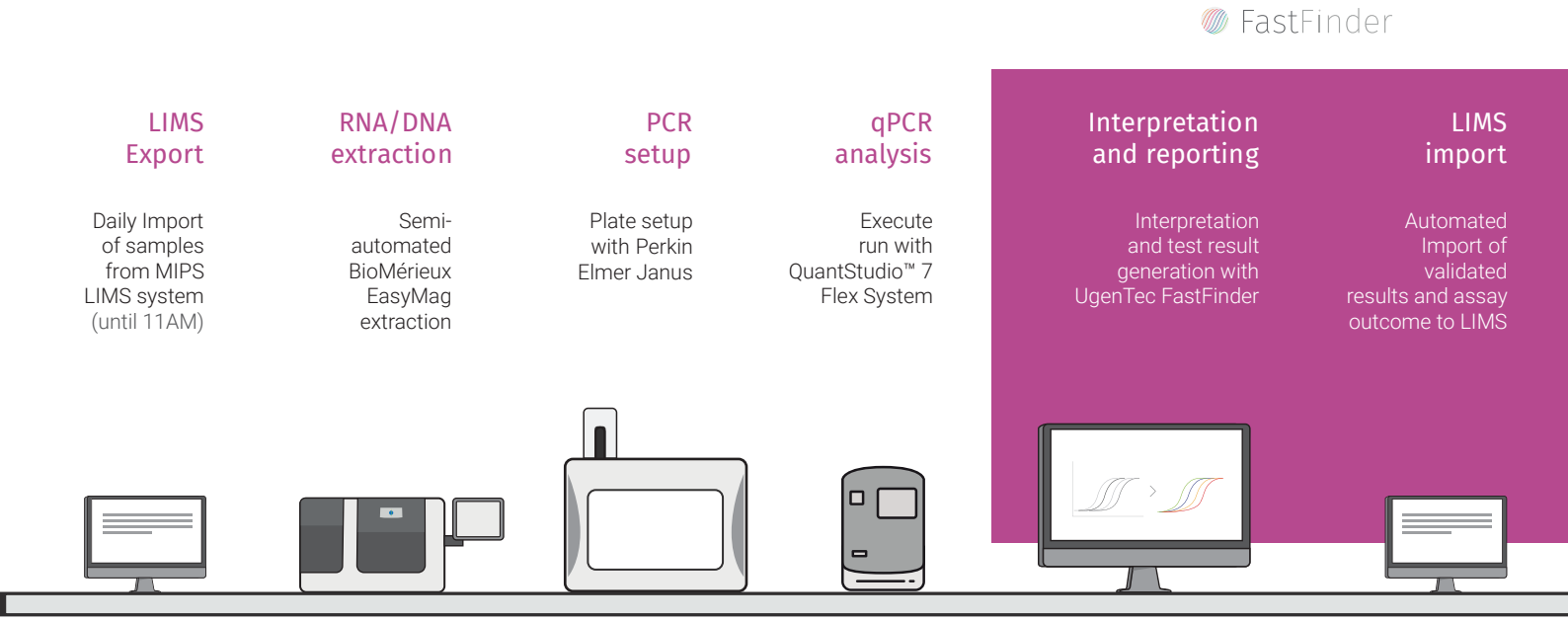
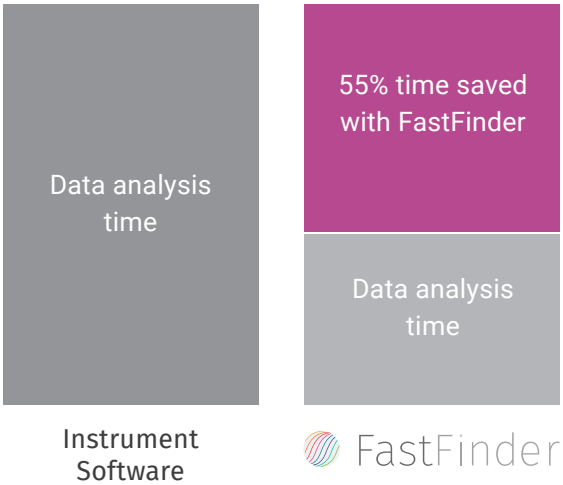


Fig. 1
Operations at OLVZ Aalst molecular laboratory feature automation across the workflow.



Time spent on real-time PCR analysis & processing of results

Challenges with data analysis, interpretation and reporting

Challenges with manual analysis

1		Result accuracy and operator dependency
2		Longer time-to-result through unnecessary curve checks
3		One-size doesn't fit all tests
4		Lack of standardisation across multiple instruments

Challenges with manual data management and reporting

1		Laborious and error-prone manual data entry
2		Interpretation complexity for multiplex assays
3		Lack of LIMS integration
4		Time lost on verification
5		Building and accessing a lab database

Challenges with data analysis, interpretation and reporting

» *When the lab looked into automating and streamlining the routine diagnostic qPCR workflow, the main areas of improvement were in the traditional analysis workflow and limitations that come with instrumentation software; challenges with the manual process of generating and signing off on assay reports; and the need to track assay, sample and run QC in a robust manner.*

Challenges with manual analysis

CHALLENGE 1 - Result accuracy and operator dependency

Amplification plots are generated by the software of the PCR device. However, the algorithm underlying the device software is not always correctly evaluating the signal. Moreover, the baseline is set automatically by the PCR instrument software and is influenced by the noise at the beginning of the cycles. It is possible to manually set the threshold, but that leaves the door open for subjectivity - introducing operator-dependent bias in results.

> **IT IS POSSIBLE TO MANUALLY SET THRESHOLDS, BUT THAT LEAVES THE DOOR OPEN FOR SUBJECTIVITY**

CHALLENGE 2 - Longer time-to-result through unnecessary curve checks

The interpretation of the amplification curve is done per target. This leaves calling results across multiple targets a manual, time-consuming and potentially error-prone process. Secondly, instrument software will require individual investigation of each and every curve. For example, when running a batch of 10 samples and 2 controls for a respiratory analysis, the operator will check every curve separately, regardless of whether a correct call was made, there was amplification or not, or the data looks unusual. To optimize time to result, a solution is needed to automate the bulk and only look at exceptions.

CHALLENGE 3 - One-size doesn't fit all tests

As the cycler software has no knowledge of the test, aspecific signals cannot be defined and recognized by the software that comes with the instrument.

CHALLENGE 4 - Lack of standardization across multiple instruments

Labs often have diversified qPCR instrumentation. Each instrument comes with its own software, which means operators have to be trained on each of them. Moreover, the **software that comes with instrumentation** universally **lacks user friendliness, configurability, automation capabilities** and **anything more than basic interpretation** support.

Challenges with manual data management and reporting

CHALLENGE 1 - Laborious and error-prone manual data entry

In a non-automated setup, an operator will need to print out the Cq values resulting from the assay. Manual data entry to a worklist needs to be done per assay, which takes time and introduces error risk. For some labs, this is tedious data entry into an excel sheet, for other labs, this happens with pen and paper. Both methods are time consuming and error prone. Moreover, manually verifying where samples are located

> **"** INSTRUMENT SOFTWARE OFTEN LACKS USER FRIENDLINESS, CONFIGURABILITY, AND AUTOMATION CAPABILITIES

on a plate is another time-consuming and repetitive task that takes time away from activities that actually add value.

CHALLENGE 2 - Interpretation complexity for multiplex assays

Especially for more complex assays, such as multiplex assays and assays with above-average complex instructions for interpretation, overall conclusions need to be generated based on multiple inputs.

CHALLENGE 3 - Lack of integration with LIMS

Another time consuming and error prone component is manually introducing the results in the LIMS system. Some labs build bespoke spreadsheets and copy-paste values to feed them into the LIMS, with every copy-paste step allowing for errors to happen.

CHALLENGE 4 - Time lost on verification

With manual interpretation, confirmation of results by a clinical biologist is required. An additional pair of eyes is needed to check transcription errors. Note that whether interpretation is automated or not, it is still necessary to perform a clinical validation.

CHALLENGE 5 - Building and accessing a lab database

When multiple instruments, pieces of software and lab IT systems are in place, it is often tedious to go back to earlier results. While basic information such as run details and sample identifiers are usually managed in a LIMS, more detailed queries are harder. Examples include accessing all historical curves to distinguish weak from strong positives, tracking assay performance over time, and comparing QC metrics across runs. The availability of prior results in one central place, with all necessary details and appropriate query capabilities, saves significant time over manual lookups or extracting information from the LIMS, which is often not geared at molecular biology data management.

Automating data analysis, interpretation and reporting: taking the analytical workflow full circle

Molecular diagnostic labs are confronted with an ever-increasing workload. With a rising number of samples and analyses per day, more work needs to be performed with the same number of people as highly-trained personnel is expensive and increasingly difficult to attract and retain.

What's more is that lab operations are becoming more complex. Labs develop larger test menus, increased catchment areas and lab business development lead to an increased number of assays. This constant evolution of methods and continuous expansion of diagnostics puts additional pressure on team, infrastructure and operations.

To address these challenges, OLVZ Aalst adopted **FastFinder**. **FastFinder** is a software platform for data analysis, interpretation support, and reporting for qPCR assays that makes life in the lab easier.

FastFinder key benefits

1 Facilitates interpretation

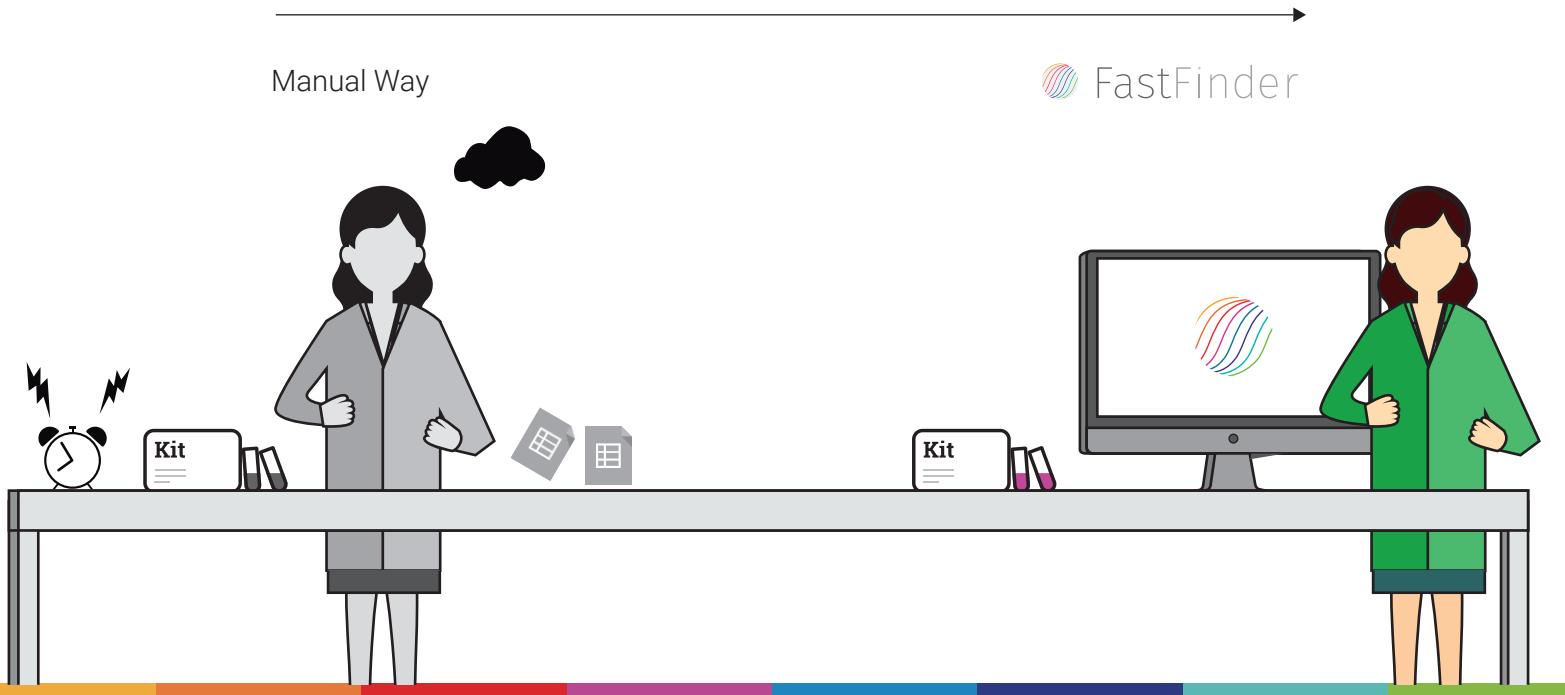
The platform uses artificial intelligence to analyse raw PCR data from multiple commercial PCR devices. Combined with smart decision logic for combining results from targets and controls into an actual assay result, labs reach a standard assay interpretation, highly accurate results, and almost no eyes-on time.

2 Automates the routine lab interpretation and reporting workflow

FastFinder can go from sample readout to result in only a few clicks, dramatically improving quality & decreasing the overall time-to-result, effectively allowing laboratories to automate their workflow with a software that can analyze curves exactly like experienced laboratory scientists.

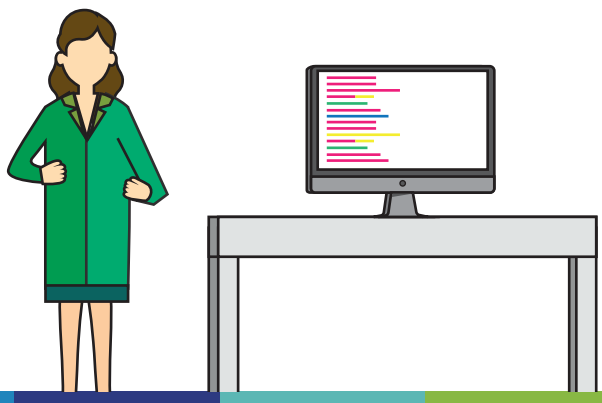
3 Ensures QC traceability

FastFinder can track assay, run, lot and instrument QC metrics over time, visualize them in a range of appealing and easy to read plots such as Levey-Jennings control charts, and automate the application of your Westgard rules of choice - an industry standard for quality control (QC) practices.



The FastFinder solution at OLVZ Aalst: Software key Features

1. Increased accuracy of test outcome with smart curve calling
2. Complex test result automation
3. Manage multiple tests with ease
4. Flags exceptions for manual review
5. Automated reporting
6. An archive and queryable database at your fingertips



The FastFinder solution at OLVZ Aalst

» Fast and accurate interpretation

The FastFinder platform offers intelligent algorithms for curve calling, and powerful tools such as Decision Trees that take away the manual work on calling test results.

FEATURE 1

Increased accuracy of test outcome with smart curve calling

The FastFinder software relies on Machine Learning to optimize the assessment of curves. Drawing from Artificial Intelligence techniques, FastFinder is able to go beyond simple thresholding, and use more complex features of a curve such as angles and slopes, noise measures, and more complex models under the hood. These trained algorithms then **detect target amplification intelligently**, increasing the accuracy over manual evaluation using instrument software. This standardized interpretation support **reduces errors** and saves time by removing the need to manually assess the bulk of the curves.

> **“ LABS CAN ADOPT INCREASINGLY COMPLEX TESTS WITHOUT INCREASING THE RISK OF ERRORS OR REQUIRING EXTENSIVE LAB SCIENTIST AND MOLECULAR BIOLOGIST TRAINING.**

FEATURE 2

Complex test result automation

While smart algorithms trained on millions of curves and hundreds of assays are a powerful tool underlying **FastFinder**, its automation power doesn’t stop there. Once curves are called, FastFinder will automatically call Positive and Negative result status (e.g. “Positive for influenza A”) by implementing the assay’s Instructions for Use. With so-called “embedded decision trees”, these procedures process Cq cut-offs, how to combine different targets, how to deal with outliers and invalid controls, and how to finally call presence of a specific pathogen. This removes the need to be executed manually or via error-prone spreadsheets running complex macros. In this way, **labs can adopt increasingly complex tests** without increasing the risk of errors or requiring extensive lab scientist and molecular biologist training.

FEATURE 3

Manage multiple tests with ease

Labs deal with an increasing menu of tests with higher levels of complexity. FastFinder manages different tests in a clear, convenient and structured fashion. Through Assay Plugins (which combine assay information such as targets, channels and device-specific information, an intelligent curve calling algorithm, and the assay's decision tree) labs can run their multiple test workflows on a single software platform and database.

FEATURE 4

Flagging of exceptions for manual review

Instead of looking at all the wells, curves, and samples on a run, the software will drastically cut hands-on time by detecting exactly those anomalies that require further investigation. When a run is analyzed in FastFinder, the user can quickly glance at the overall software-interpreted result, check control validity or whether any QC violations occurred, and use the user-friendly interface to check any individual result and compare them with their respective controls if desired. In cases where the algorithm is unsure of its classification, results are flagged for lab technicians to assess the result manually, before an analysis is authorized for send-out.

»

Did you know?

With **FastFinder**, every assay gets a performance specification sheet. For example, when an Assay Plugin (combining the algorithm and the Instructions for Use) is trained and validated, the sensitivity and specificity, alongside a number of additional performance parameters, are determined and documented.

This documents how narrow the so called *grey zone* can be defined, and how few samples (typically single digit numbers out of a hundred curves) are expected to require manual review.

FEATURE 5

Automated reporting

FastFinder will generate a final PDF or CSV report and even automatically push results to your LIMS system. Reduce paper waste, reduce time preparing reports, and most importantly, reduce the error risk: no more manual transcription. Instead, FastFinder brings a standardized generation of overall conclusions and a direct transfer to the LIMS. Complemented by an automated audit trail, keeping track of lab decisions and exceptions, this makes for a robust and compelling workflow.

FEATURE 6

An archive and queryable database at your fingertips

There is significant value in keeping a historic trail of all your analyses in one single database. Not only can you go back to any analysis from any point in time from a central repository, you can also run smart queries - for example, "show me all positive assays for test x during time period y", "show me all failed tests on instrument z", and much more.

A key example is discerning **weakly vs. strong positive results**. Separating those types of results **is essential to support the lab's assay validation efforts**.

Having a single archive across all instruments, for all tests that are on the lab's menu, and for every operator and scientist, even across multiple sites if the lab is distributed geographically, is a tremendous asset.

> **WITH ONE SINGLE ARCHIVE ACROSS INSTRUMENTS, TESTS AND TECHNICIANS, LABS CAN RUN SMART QUERIES ACROSS THEIR ENTIRE DATABASE.**

Looking for specific samples of interest, preparing for an audit, reporting on data analysis turnaround times and efficiency, looking for instruments that are performing sub par, or queries for new test research, such as "show all Enterovirus samples for validation of a new test" are only a few example uses.

Of note is that all the relevant data are available right from FastFinder. For example, when a user wants to revisit the curves, or drill down on results, or see whether positives were strong or weak, ... these details are readily available. Not so with LIMS systems, since they are usually generic and not optimized for common use cases in molecular biology.

Robust and informative QC

The QC features of the FastFinder platform specifically are beneficial especially in a routine diagnostic context:

ACCESS QC INFORMATION IN REAL TIME

As you are analysing a run, you can already assess the positive control. This avoids the need to switch screens to other pieces of software, or wait for QC reports from more remote lab IT systems or systems that are shared with chemistry and serology assays, and sometimes only generate reports well past analysis time.

FULL CONTROL OVER QC

Especially when labs run LDTs, you have control over which controls get followed up in the QC module. For example, you can separately track the internal control, the extraction control, the negative control, etc.

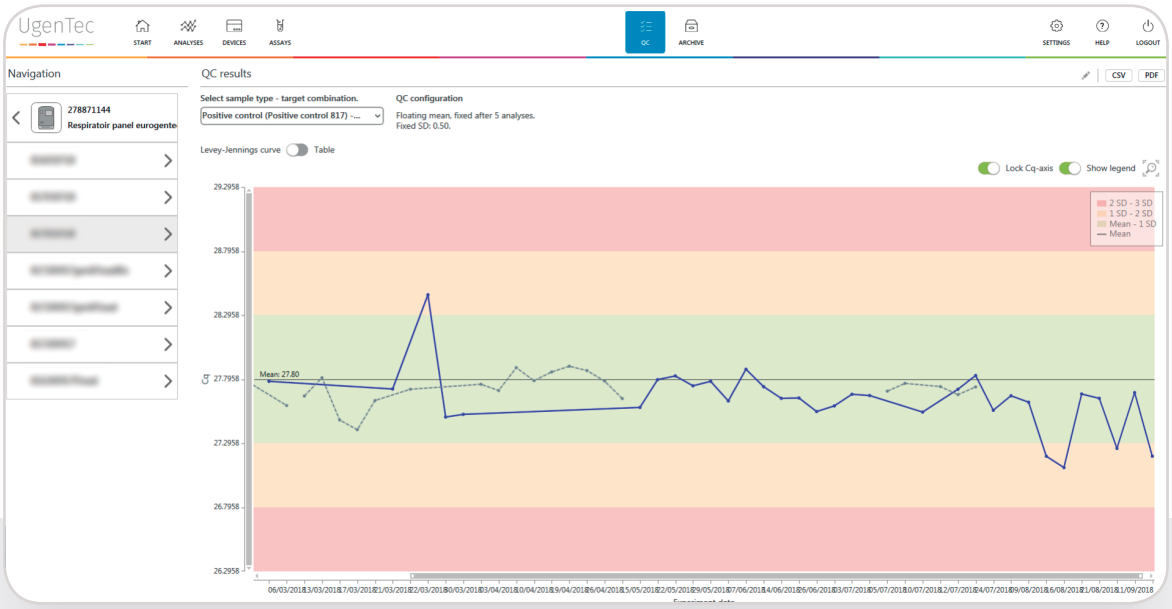


Fig. 3
An overview of how a positive control evolves over time. The Cq value is plotted on Levey-Jennings bands, allowing to apply rules and flag QC exceptions in real time.

Use case: a complex, lab-developed syndromic respiratory assay

» OLVZ runs an in-house respiratory panel comprising 25 targets in 8 multiplexes. In a routine setting, each respiratory sample is screened for the full respiratory panel.

Automation opportunities span the pre-analytical (accessioning, sample preparation, plate setup), analytical (running the actual assay on the instrument) and post analytical (data processing, analysis, interpretation, reporting). OLVZ has taken up automation opportunities across the board. For example, in addition to adopting FastFinder for the post-analytical portion of the workflow, the same qPCR conditions apply for all multiplexes, making for a standardized protocol. Also, OLVZ works with pre-filled (primers and probes) 96 well plates or strips.

PCR	TARGET
1	RSV (2 targets), hMPV, RNA IC
2	influenza A (2 targets), influenza B
3	adenovirus, bocavirus, PhHV IC
4	PIV2, PIV3, enterovirus
5	PIV1, 4, rhinovirus
6	coronavirus OC43, NL63, HKU1 en 229E
7	*C. pneumoniae, M. pneumoniae, L. pneumophila*
8	*B. pertussis, B. parapertussis, B. holmesii, 16S Bordetella*

Table 1.
Overview of the different targets included in the respiratory panel.

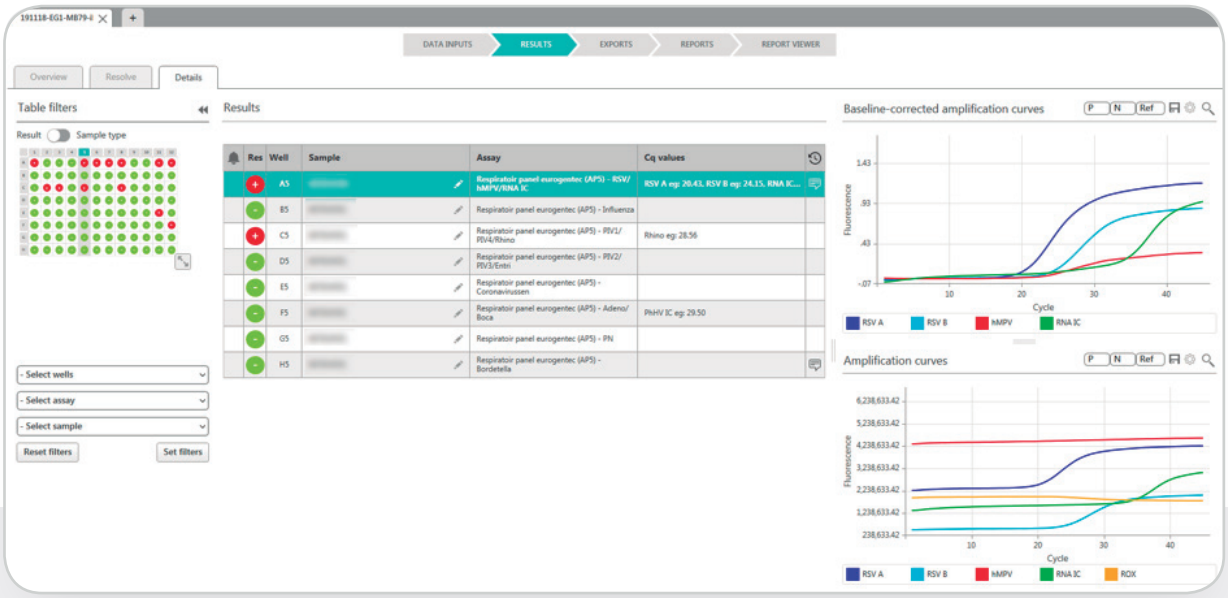


Fig. 4
This screenshot shows the detailed view where individual wells, curves, and overall plate setup are visible. This view allows for an easy drill-down in pre-result details.

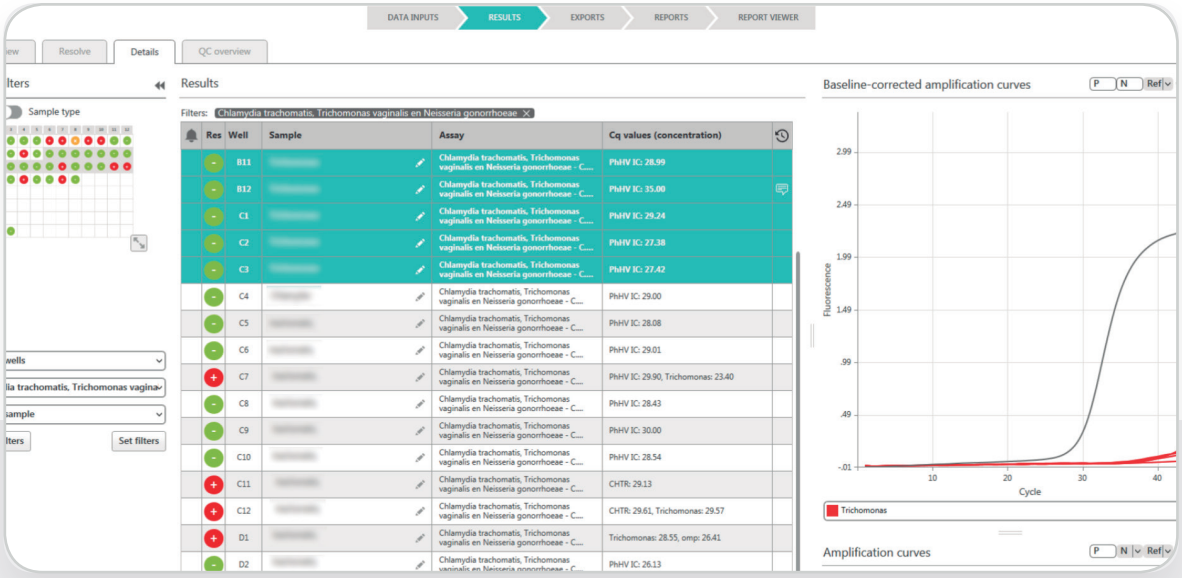


Fig. 5
Although the workflow is largely automated, it's not a black box. Users still have access to data visualisation.

Q&A with the lab

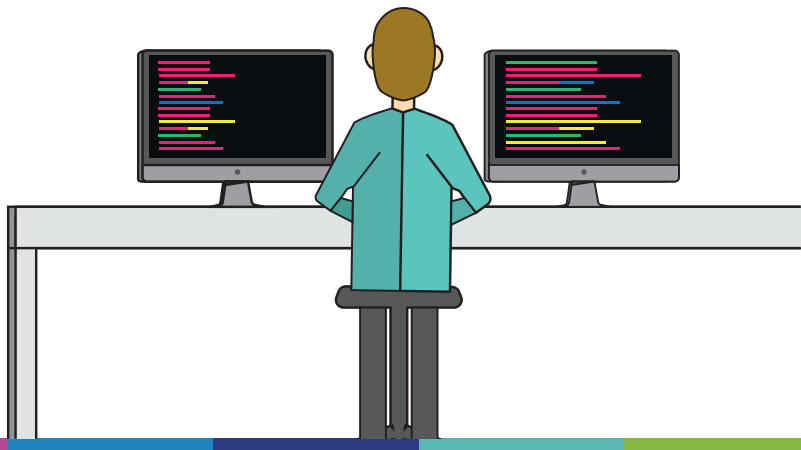
» *We talked with Karen, from the OLVZ Lab and she discussed her experience using FastFinder*

Q - Why did you decide to develop or adopt an LDT, and what role does software play in the process?

A - A well-designed in house assay gives us the advantage of knowing exactly what is detected and allows us to adjust the assay according to seasonal variations and mutations in virus strains. This flexibility is very important in molecular diagnostics and can be achieved quickly when using in-house assays.

In addition to that, bringing an assay in house is a cost-saving effort for the lab, especially when test volumes are significant. There are 2 caveats with LDTs. First, you need to invest time and effort in developing the primers and probes. Secondly, for complex assays, the burden can shift from consumables and reagents (compared to commercial off-the-shelf kits) to additional time spent on analysis and interpretation. This is why interpretation support software needs to be part of the equation.

“ **FOR COMPLEX ASSAYS, THE COST-BURDEN CAN SHIFT FROM CONSUMABLES AND REAGENTS (COMPARED TO COMMERCIAL OFF-THE-SHELF KITS) TO ADDITIONAL TIME SPENT ON ANALYSIS AND INTERPRETATION. THIS IS WHY INTERPRETATION SUPPORT SOFTWARE NEEDS TO BE PART OF THE EQUATION.**



Q - Is automation of curve calling the most compelling reason to adopt FastFinder?

A - It's just one aspect. **FastFinder** brings much more: apart from the algorithm, the decision tree also plays an important part in the facilitation of result interpretation. Especially for assays such as our respiratory panel, because it consists of 25 targets + 2 internal controls in 8 mixes, all with their specific inter- & intra-mix logic. So while the trained algorithm to do smart curve calling is a boon, the true time gain lies in the assay automation, from sample to result instead of from sample to curves.

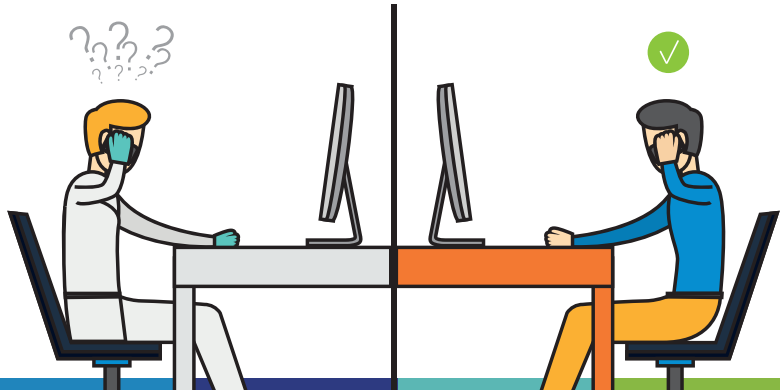
Q - Do you have other assays in development that can benefit from this method, and how universal is the approach?

A - We are in the process of developing additional assays, and we are currently in the process of validating them. The respiratory panel is one of the first assays we have released into routine production.

Q - What is one of the most compelling reasons to standardize your analysis on FastFinder?

A - In multiplex assays, operator experience is needed to identify false positives: identifying cross talk and background signal can complicate the analysis. This requires significant experience and training of personnel.

With **FastFinder**, this training comes out of the box. **FastFinder** is trained on a data set that is based on calls made by an experienced lab scientist, and this experience is represented in its model, in a reproducible fashion. Having this locked down in the software means that you no longer have to train new lab personnel. Of course you still need a molecular biologist that is trained to look at the cases that need manual verification, and take decisions for the curves in the "resolve" tab, but the time gain is significant.



Did you know?

The **FastFinder** platform "locks down" all the assay-specific logic in an *Assay Plugin*. This makes for a standardized, uniform approach to interpretation support - the curve calling is specific to the assay, the logic of combining channels, controls and individual marker outcomes is fixed.

What's equally powerful, is that this model also supports *updates* to assays. As more data becomes available, and as the logic of an assay gets refined over time, the model can be re-trained, and a next version of an Assay Plugin can be made available. Then, once the validation based on existing data shows at least equivalence, the new and improved assay can be deployed and used.

Q - What are the Decision Trees in the software exactly, and why are they important?

A - Decision trees represent the interpretation rules - or the SOP - for your test. For example, what do you do if a positive control isn't positive? What do you do when a result depends on a complex combination of statuses on individual channels? For Bordetella, for example, how do you avoid to manually have to check that 16S is positive, together with the *B. pertussis* channel, to conclude *B. pertussis* is positive? This manual work can be avoided. This is what Decision Trees do in the software: they represent your interpretation rules, and take the manual work and error risk out of them.

Q - How often do you still have to manually intervene? What is the performance of the respiratory assay?

A - The performance is documented during the validation of the assay. For our respiratory panel specifically, which is validated on the QuantStudio 7, the performance parameters are as follows. In digging deeper, we learned that over 103.059 curves, the average accuracy is:

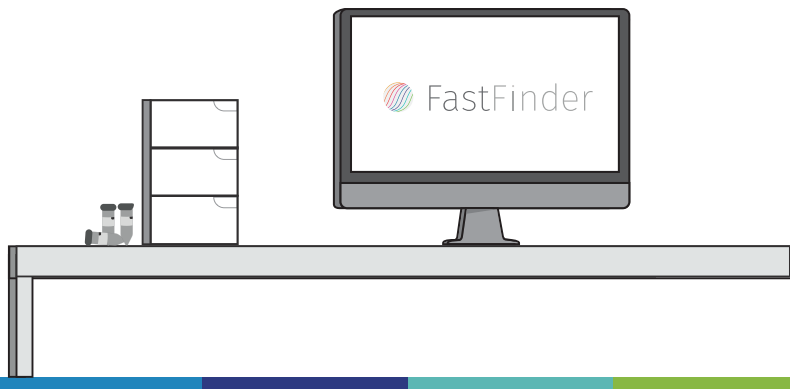
PARAMETER	VALUE
Balanced Accuracy	99,78%
True Positive Rate (Sensitivity)	99,82%
True Negative Rate (Specificity)	99,74%
Positive Predictive Value	1,49%

Q - How many assays are run per month for the respiratory panel? What time saving do you estimate is brought by FastFinder?

The volumes are seasonally dependent. During winter time, we run around 1000 samples per month. During summer the volume drops to around 150 samples per month. We estimate that implementing **FastFinder** into our routine workflow has allowed us to more than double the number of assays we can run in the same amount of time. To be precise, our assessment shows a 55% time gain.

"We estimate that implementing FastFinder into our routine workflow has allowed us to more than double the number of assays we can analyze in the same amount of time."

- Karen Dierickx, molecular biologist at OLVZ Aalst



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