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FastFinder Analysis: Increased standardization of data analysis of multiplex PCR for non-invasive prenatal RHD testing.

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FastFinder Analysis: Increased standardization of data analysis of multiplex PCR for non-invasive prenatal RHD testing.

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> There is real risk for errors if you simply accept cycler software calls at face value. You either need to add a time-consuming manual review step, or adopt intelligent software like FastFinder Analysis.

What you'll learn:

- How a blood bank molecular laboratory automated non-invasive prenatal Rhesus (NIP RHD) testing uses FastFinder Analysis for interpretation and reporting to standardize lab operations
- How OUH cut qPCR analysis time by removing a secondary result verification step
- How technician variation can be brought to all-time lows even for complex workflows
- How to eliminate the error risk that comes with manual data entry

Introduction to the workflow at Odense University Hospital

The Department of Clinical Immunology at the Odense University Hospital covers a population of 1M+ people in the region of Southern Denmark. The department is grouped into five different labs, that is focused on testing for blood banking and Clinical Immunogy.

The molecular biology lab offers a variety of different test including next generation sequencing (NGS) for immunodeficiency patients, HLA typing for amongst other stem cell transplantations & genomics for blood typing incl. noninvasive screening for fetal blood groups. The lab processes more than 6,000 samples per year.

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Dr. Marianne Jakobsen has been with the hospital for 20 years, leading a team of four technicians and overseeing the laboratory operations. The laboratory is equipped with an Ion GeneStudio S5 for NGS, two ABI 3500's for Sanger sequencing, several Roche LightCyclers, a Roche MagnaPure LC2.0 for extraction, several benchtop PCR machines and a Luminex 200.

Non-Invasive prenatal RhesusD testing (NIP RHD)

The diagnostic test automated in this collaboration is the NIP RHD. Haemolytic disease of the fetus and the newborn (HDFN) is caused by RHD incompatibility between the fetus and the mother and is a condition where D antibodies in a pregnant woman's blood destroy her baby's erythrocytes. The RHD gene consists of ten exons and is highly polymorphic with more than 350 detected variants that determine the expression level of D-antigen on the surface of erythrocytes. Most Caucasian RHD-negative persons lack the whole RHD gene. The cases of HDFN have been significantly reduced by anti-D prophylaxis that was implemented in the late 1960s. Since 2010, it has been mandatory to test all RHD-negative pregnant women in Denmark before the administration of anti-D prophylaxis to avoid unnecessary treatment. The presence of cffDNA circulating in the maternal blood has made it possible to work through a non-invasive sampling method using the maternal plasma.

OUH deploys an in-house multiplex PCR test in which they test all samples for the following three targets: exon 5 (VIC channel), exon 10 (FAM channel), and CCR5 as an internal control (Cy5 channel). All samples are tested in triplicate starting from cffDNA.

To ensure a qualitative process, the laboratory takes into account the results of a negative water sample which is tested four times, a duplicate negative RHD sample which must be negative for both exons 5 and 10, but positive in the CCR5 channel and a triplicate positive Plasma control which must be positive for the three targets. Three positive samples with a known concentration starting from 0,004 ng/L to 0,4 ng/L are tested in duplicate in the same run and provide key information on the performance of the test.

Depending on the number of positive replicates for exons 5 and 10, an overall result is generated. Further examination is necessary for samples that have Cq values for the RHD targets below 33 because this may represent a maternal RHD variant that would conceal the fetal RHD genotype.

Workflow overview

The laboratory uses a host of technologies to run its operations efficiently

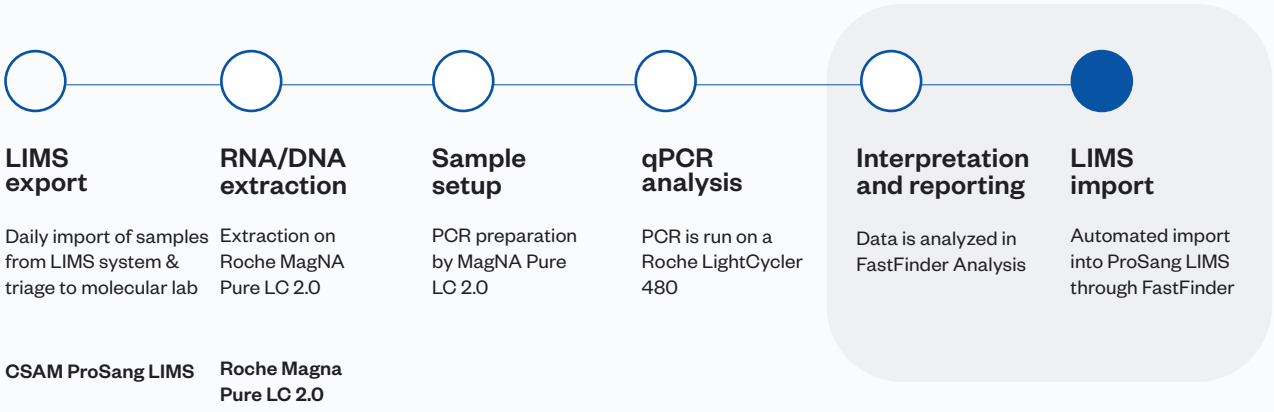

Figure 1 - Operations at Odense University Hospital feature automation & standardization across the workflow.

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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 lay in the traditional analysis workflow and in 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.

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Challenges with manual analysis

Challenge 1

The complexity of triplicate testing. Subjecting every sample to triplicate testing solidifies OUH's QA efforts, but also increases complexity in their routine workflow.

Challenge 2

Wrong results when using cycler software without manual review. The Roche LightCycler data analysis software would sometimes provide erroneous results - for instance, flat curves with a Cq value of 26. This forced OUH to check every curve manually, introducing technician bias and manual work.

Challenge 3

Variable results across technicians. The manual checking of curves and manual execution of standard operating procedures introduced a variation in data analysis and interpretation between technicians.

Challenge 4

Time-consuming manual data entry. Before, data entry was largely a manual process and included tedious double entry methods that absorbed time and left room for transcription errors.

A blood bank that is ready for a high throughput future

With more samples to process each day, and an increasing regulatory burden with the advent of new CE IVDR regulations, the workload of the molecular laboratory is going up with the same number of people to do the job. When NIP RHD testing became mandatory in Denmark, Odense developed a robust lab-developed test for screening across the entire Region of Southern Denmark. Now that the test has been in use for over ten years, volumes are increasing and regulatory pressure from the new IVD Regulation is growing. Armed with these insights, the laboratory in Odense decided to implement FastFinder Analysis for their LDT. Key reasons for migrating away from the manual analysis to FastFinder include:

- **To facilitate interpretation,** the platform uses artificial intelligence to analyze raw PCR data from multiple commercial PCR devices. This intelligence can accurately classify curves, eliminating the need for multistep curve checking. Coupled with smart decision logic for combining results from targets and controls into an actual assay result, labs reach more reliable measurements, highly accurate results, and almost no eyes-on time.
- **To automate 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 OUH to automate their workflow with a software that can analyze curves exactly like experienced laboratory scientists.

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The FastFinder solution at Odense University Hospital

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.

> Trained algorithms detect target amplification intelligently, increasing the accuracy over manual evaluation using instrument software

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 even much 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 interpretation errors and saves time by removing the need to manually assess the bulk of the curves.

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 RHD") by implementing the assay's Instructions for Use. FastFinder Analysis has decision trees that take away the need for manual interpretation and complex spreadsheet macros. Decision trees describe how the software has to automate the steps in the assay's Instructions for Use. Such tasks include decision-making based on positive and negative controls, Cq cutoffs, intelligent combination of different targets, how to deal with outliers and invalid controls, and how to finally call the assay result, determining the presence of a specific pathogen or gene. In this way, labs can adopt increasingly complex tests without increasing the risk of errors or requiring extensive lab scientist and molecular biologist training. This approach is especially beneficial for multiplexed assays, as well as multi-well tests.

Feature 3

Automated reporting.

FastFinder will generate a final PDF or CSV report and even export 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 to keep track of lab decisions and exceptions, this makes for a robust and compelling workflow.

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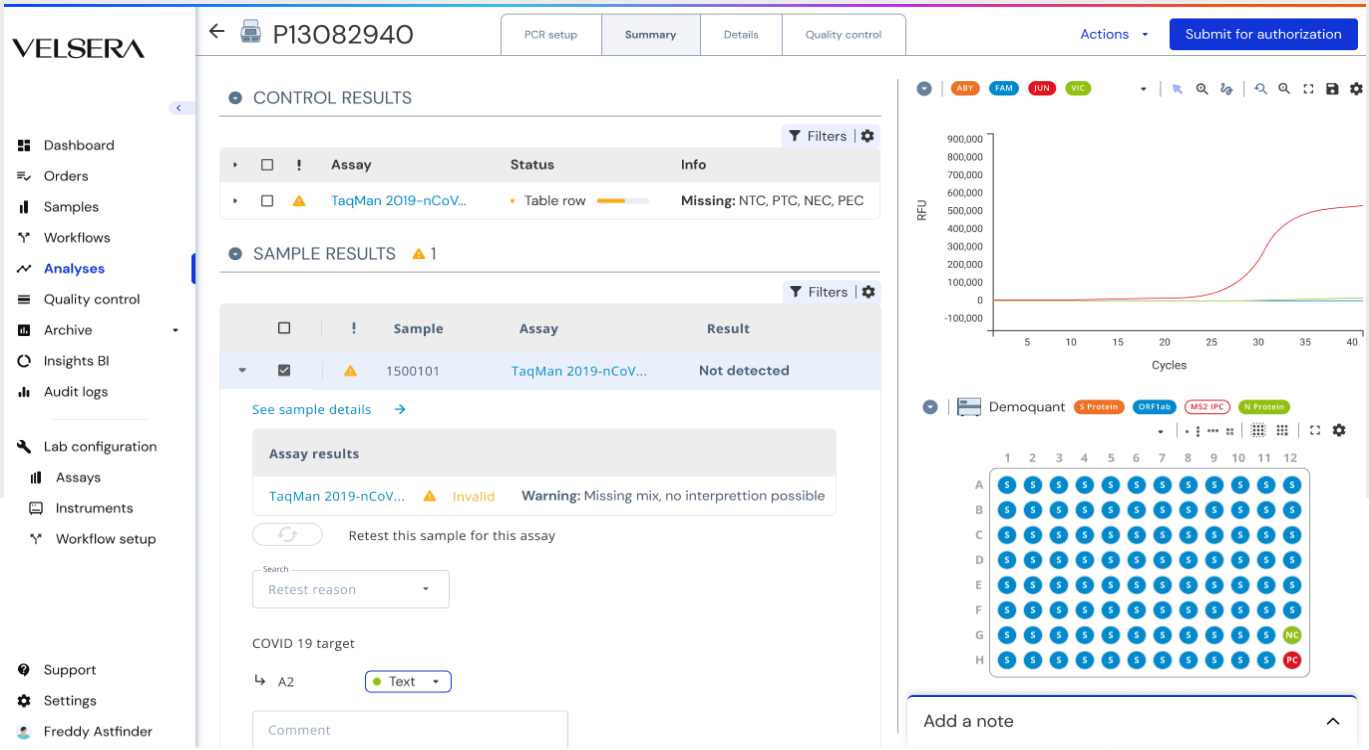
Use Case: Non-invasive prenatal Fetal Rhesus Disease testing

Great features for any workflow

Rhesus disease is a condition where antibodies in a pregnant woman's blood destroy the fetus' blood cells. The condition does not harm the mother, but does cause anemia and jaundice. The disease occurs when the mother is RHD-negative and the baby is RHD-positive. Anti-D immunoglobulin is a proven cure and the disease is virtually extinct due to 50 years of prevention.



Why did you decide to develop an LDT?

Commercial assays are nice, but in our current reimbursement system, implementing them would not be viable. With our own test, we have a one time investment cost, but can spread that over many years. The primers and probes are affordable and our test was designed in a robust way, allowing us to save lots of money for the healthcare system.

What about the new IVD Regulation - will you be able to keep up with those new regulations?

We already have a project running to further standardize our operations and perform clinical trials to certify our methods. We have enough samples to make a business case, and already have standardized methods such as extraction on MagNA Pure XL and data interpretation on FastFinder Analysis. For our lab-developed test, overcoming IVD Regulation should be feasible.

What's the most important FastFinder aspect for your laboratory?

Two things really make our laboratory workflow better. For one, I now maintain a fully automated data analysis suite. The automated analysis is nice, but I can still peek below the radar and check the data if I feel the need to. This is the kind of automation that is perfect for molecular laboratories, automation when possible, user intervention when required. The other part is the automated data connections. Although we're still waiting for some final implementations on our LIMS side, we expect to see a significant time saving and increase in standardization.

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> FastFinder's level of automation is perfect for molecular laboratories: automate where possible, allow user intervention where required.

What about QC - what are some of the metrics you track across the laboratory?

Our QC is currently implemented through large spreadsheets. During the training, which was given very promptly after requesting it online, we learned about the automated QC module. In the future, we will track our QC in this automated tool within FastFinder Analysis.

Can you shed some light on the performance of the software versus the manual analysis workflow?

During validation of our pipeline, we documented and tested about 3,515 wells (with 10,545 different curves in total) retroactively. The data analysis method deployed in FastFinder can easily replace our technical result validation.

Parameter	Value
Balanced Accuracy	99.76%
True Positive Rate (Sensitivity)	99.56%
True Negative Rate (Specificity)	99.96%

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