FastFinder: simplifying data analysis for lab-developed syndromic TaqMan® Array Card panels

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> “ Standardized multi-target interpretation on FastFinder makes life easier. It enables us to structure the large amount of data generated by our lab-developed TaqMan® Array cards, facilitating interpretation
>
> - Patrick Descheemaeker, AZ Sint-Jan Brugge

What you will learn

In this case study, you will gain insights into:

- Strategies for setting up syndromic lab-developed panels on a high well count platform
- How software automation standardizes data processing and reduces the risk of interpretation error
- how automated QC tracking allows for real-time intervention in case of quality deviations

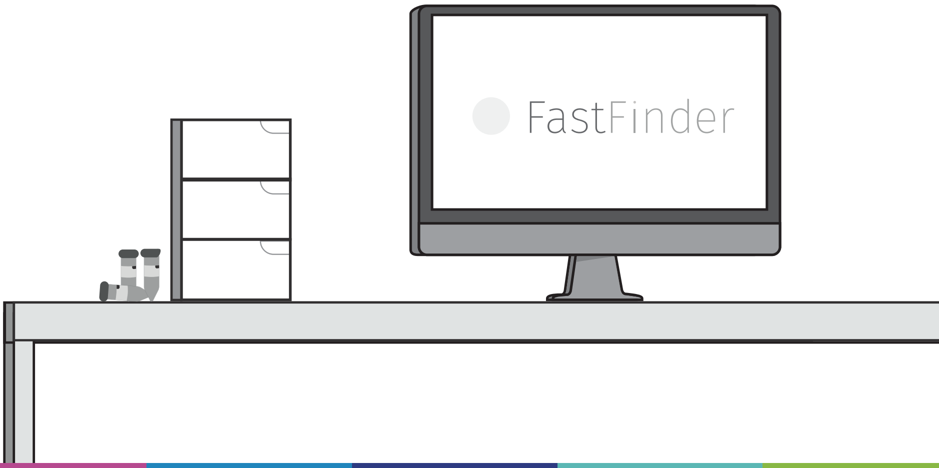
Introduction to the workflow at AZ Sint-Jan Bruges

» The Molecular Microbiology lab at AZ Sint-Jan Bruges (www.azsintjan.be) is part of a routine clinical diagnostic lab. The lab testing applications span across bacteriology, virology, chemistry, immunology and hematology. The lab has established a strong reputation for its broad syndromic approach. About 19,500 samples are analyzed by molecular techniques covering 122 different microbiological parameters, mostly in syndromic panel format, generating about 450,000 results yearly.

The department of Laboratory Medicine in AZ Sint-Jan maintains an ISO 15189 certified quality management system. The lab adheres to strict quality control across its assays, infrastructure and administration. A formal accreditation is mandatory to be amenable to reimbursement under the RIZIV-INAMI federal governmental health care system agreement.

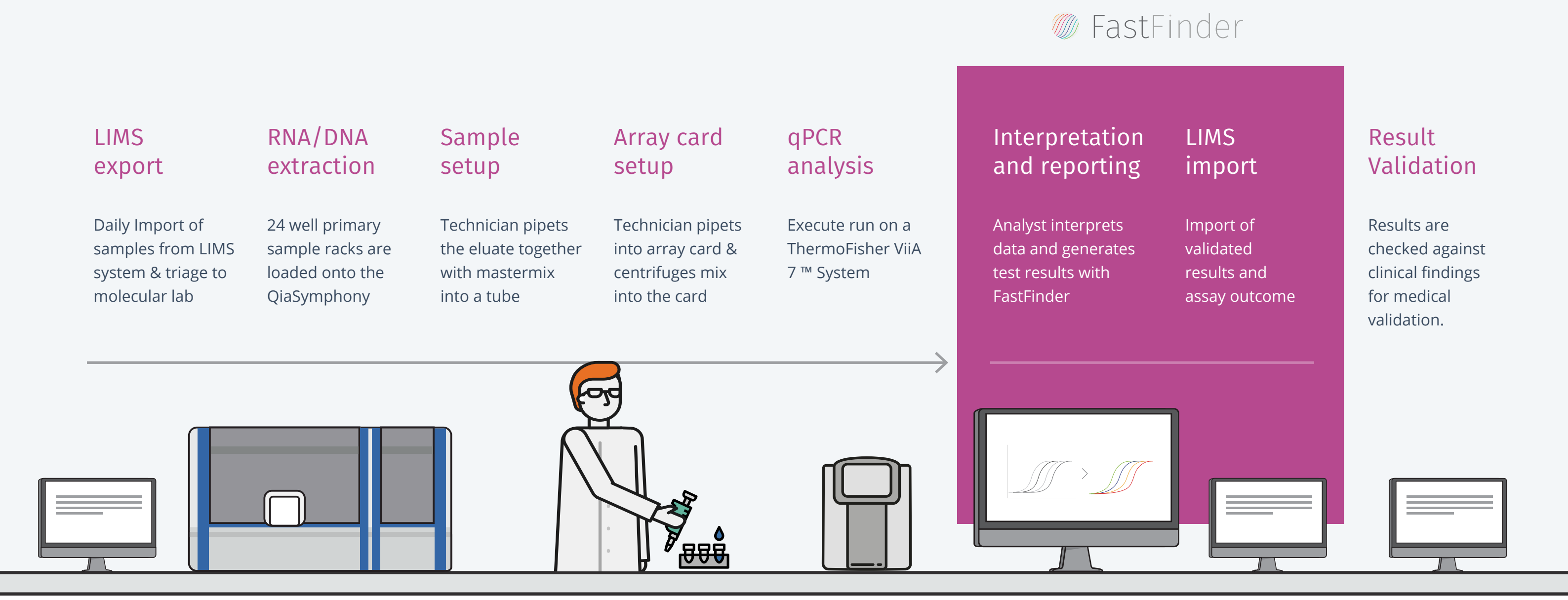
The laboratory's three main panels include syndromic analysis of sexually transmitted, gastro-intestinal and respiratory infections - all developed on Thermo Fisher Scientific custom TaqMan Array Cards. These tests were lab-developed through extensive research. They include common as well as rarer, regionally relevant pathogens.

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, and complex features to learn how to call curves with the highest confidence.



Workflow overview

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



FastFinder

LIMS export

Daily Import of samples from LIMS system & triage to molecular lab

RNA/DNA extraction

24 well primary sample racks are loaded onto the QiaSymphony

Sample setup

Technician pipets the eluate together with mastermix into a tube

Array card setup

Technician pipets into array card & centrifuges mix into the card

qPCR analysis

Execute run on a ThermoFisher ViiA 7 ™ System

Interpretation and reporting

Analyst interprets data and generates test results with FastFinder

LIMS import

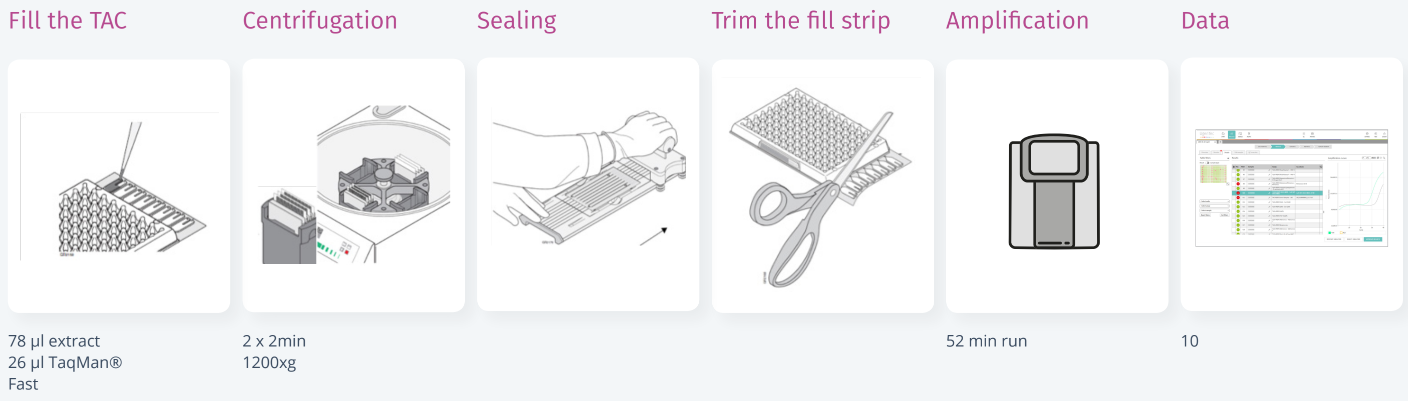
Import of validated results and assay outcome

Result Validation

Results are checked against clinical findings for medical validation.

Fig. 1
Operations at AZ Sint-Jan Bruges molecular laboratory feature automation across the workflow.

Taqman Card preparation



Fill the TAC

78 µl extract
26 µl TaqMan®
Fast

Centrifugation

2 x 2min
1200xg

Sealing

Trim the fill strip

Amplification

52 min run

Data

10

Fig. 2
Overview of the various lab operations in the routine workflow

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, reporting and signing off on assay reports; and the need to track assay, sample and run QC in a robust manner.

Challenges with manual analysis

1

The vast amount of data generated by TaqMan® Array cards.

Although highly standardized in terms of production process, the amount of data generated for a single patient sample can be overwhelming. Dividing the 384 well card format into 8 patient samples leaves for a total of at least 48 curves per patient to check and analyze (duplex PCRs not included).

2

Complex interpretation steps of the SOP for the three syndromic panels.

Once raw data has been analyzed, it's up to the operator to keep in mind the full complexity of the panel. Some pathogen combinations are not clinically relevant, although they produce perfect amplification—for example, in the Bacterial vaginosis algorithm. Multiple genetic targets per parameter must be interpreted together, some deviations in C_q values are important, others not (detecting genetic variants, sensitivity issues, reflex testing). The large number and combinatorial complexity of curves and markers makes it easy for even the trained eye to make small mistakes with significant consequences.

3

Manual entry of data into LIMS system.

Once interpreted, operators had to manually introduce results into the LIMS system. This effectively meant typing 19 to 36 rows of negative, positive, and invalid results, as well as correct C_q values for positive results for each patient sample. Manual typing of the results not only costs additional time - it is also error-prone and isn't the most exciting task for lab technicians.

A future-proof setup requires future-proof data analysis software

» 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.

Armed with these insights, the laboratory in Bruges decided to overcome many of the standardization and automation challenges by transferring the large laboratory menu to a trio of syndromic panels. One for sexually transmitted infections, one for gastrointestinal diseases and a third for respiratory diseases.

All panels were designed by the laboratory expert staff internally and implemented on the Taqman Array Cards 384 format. The cards are designed to analyze 8 patient samples at a time for a wide array of pathogens.

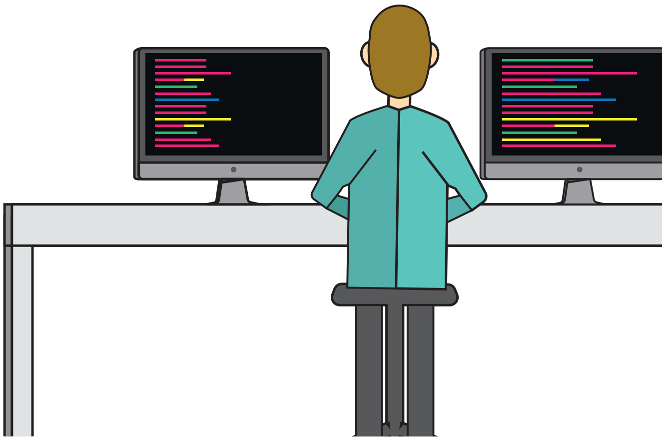
To address the workflow and analysis challenges that come with such large panels, AZ Sint-Jan adopted FastFinder. FastFinder is a software platform for data analysis, interpretation support, and reporting for qPCR assays that makes life in the lab easier.

To facilitate interpretation

To facilitate interpretation, the platform uses artificial intelligence to analyze raw PCR data from multiple PCR devices. Combined 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

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 laboratories to automate their workflow with a software that can analyze curves exactly like experienced laboratory scientists.



The FastFinder solution at AZ Sint-Jan Bruges

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.

Features

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.

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 H3N2”) by implementing the assay’s Instructions for Use.

With so-called “decision trees”, these procedures, which describe how to deal with positive and negative controls, process C_q cut-offs, how to combine different targets, how to deal with outliers and invalid controls, and how to finally call e.g. presence of a specific pathogen, no longer need to be executed.

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 assays with very high number of wells, or highly multiplexed tests.

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.

> 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

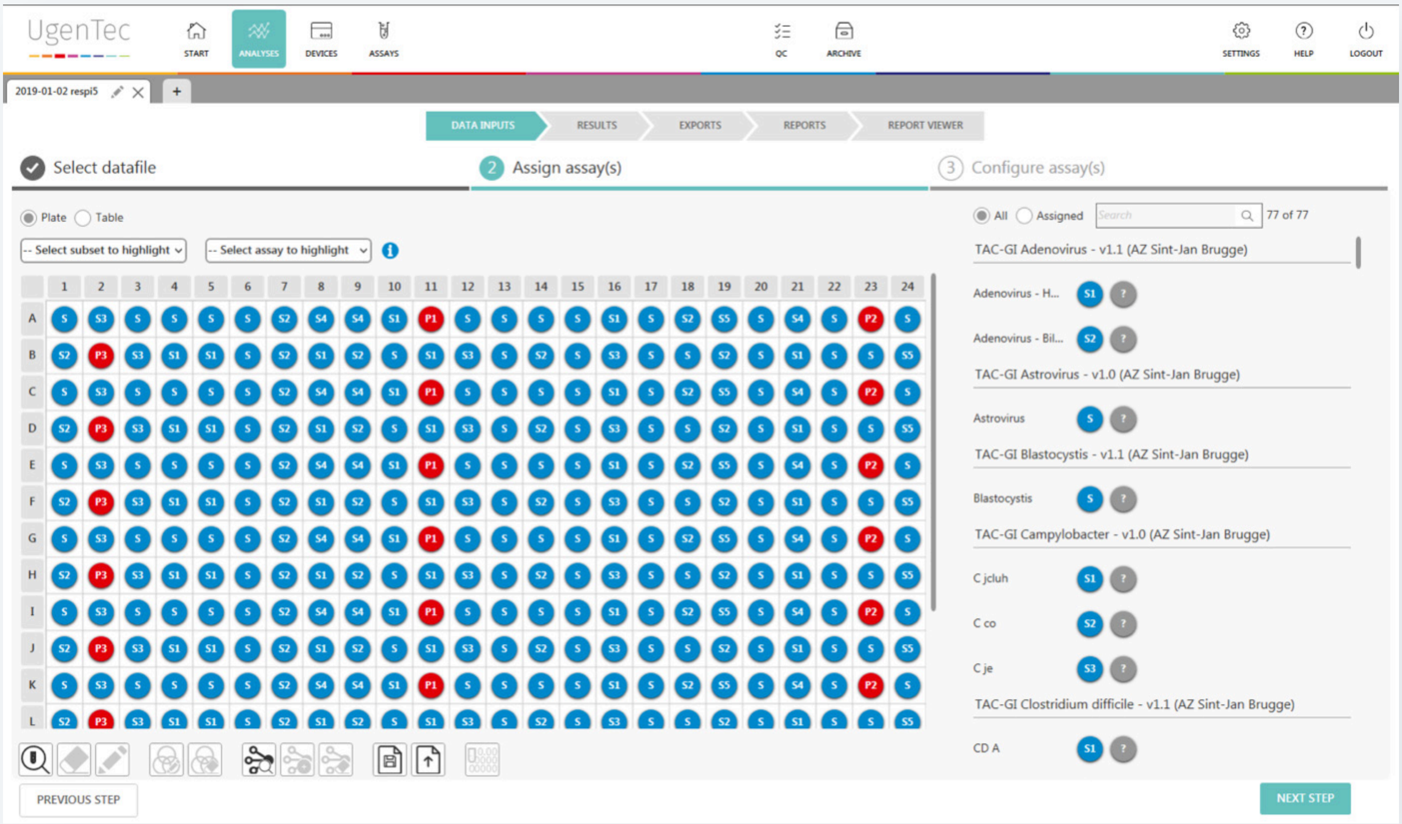
Use Case: complex, lab-developed syndromic panels

» AZ Sint-Jan runs three syndromic panels across STD's, GI and respiratory.

The STD card detects adenovirus, *A. vaginae*, cytomegalovirus, *C. trachomatis*, *C. trachomatis L serovars (CT LGV), E. coli, S. aureus, S. agalacticae, G. vaginalis, H. ducreyi*, Herpes Simplex Virus (1 and 2), *L. crispatus, M. genitalium* (and common macrolide resistant strains (A2058G, A2059G)), *M. hominis, N. gonorrhoeae, T. pallidum, T. vaginalis, U. parvum, U. urealyticum*.

The GI card detects adenovirus, astrovirus, enterovirus, hepatitis E virus, human parechovirus, norovirus (I, II, IV), rotavirus, sapovirus, *Campylobacter coli, campylobacter (non-coli/non-jejuni), Campylobacter jejuni, C. difficile (Toxin A & B)*, EA *E. coli*, EI *E. coli*, ET *E. coli*, E. *coli (O157)*, shiga-toxin producing *E. coli, Salmonella sp., Y. enterocolitica, Y. pseudotuberculosis, Enterocytozoon. bieneusi, Encephalitozoon cuniculi-E.intestinalis-E.hellem, Blastocystis hominis, Cryptosporidium spp., Dientamoeba fragilis, Entamoeba histolytica, Entamoeba spp, Giardia lamblia, Schistosoma mansoni gr, Schistosoma mekongi-S.japonicum & Strongyloides stercoralis.*

The respiratory card detects *B. holmesii, B. parapertussis, B. pertussis, B. bronchiseptica, C. pneumoniae, C. psittaci, H. influenzae, L. pneumophila, M. pneumoniae, S. pneumoniae, Coxiella burnetii, Aspergillus sp., P. jirovecii*, adenovirus, bocavirus, cytomegalovirus, herpes simplex virus, coronavirus (229E, OC43, HKU1 & NL63), enterovirus (+ subtyping of D68), influenza A (H1N1 2009, H3N2, H7N9), influenza B, human metapneumovirus, human parainfluenza virus (1, 2, 3 & 4), measles, mumps, parechovirus, rhinovirus, RSV (A & B).



● Plate ○ Table

-- Select subset to highlight

-- Select assay to highlight

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	S	S3	S	S	S	S	S2	S4	S4	S1	P1	S	S	S	S	S1	S	S2	S5	S	S4	S	P2	S
B	S2	P3	S3	S1	S1	S	S2	S1	S2	S	S1	S3	S	S2	S	S3	S	S	S2	S	S1	S	S	S5
C	S	S3	S	S	S	S	S2	S4	S4	S1	P1	S	S	S	S	S1	S	S2	S5	S	S4	S	P2	S
D	S2	P3	S3	S1	S1	S	S2	S1	S2	S	S1	S3	S	S2	S	S3	S	S	S2	S	S1	S	S	S5
E	S	S3	S	S	S	S	S2	S4	S4	S1	P1	S	S	S	S	S1	S	S2	S5	S	S4	S	P2	S
F	S2	P3	S3	S1	S1	S	S2	S1	S2	S	S1	S3	S	S2	S	S3	S	S	S2	S	S1	S	S	S5
G	S	S3	S	S	S	S	S2	S4	S4	S1	P1	S	S	S	S	S1	S	S2	S5	S	S4	S	P2	S
H	S2	P3	S3	S1	S1	S	S2	S1	S2	S	S1	S3	S	S2	S	S3	S	S	S2	S	S1	S	S	S5
I	S	S3	S	S	S	S	S2	S4	S4	S1	P1	S	S	S	S	S1	S	S2	S5	S	S4	S	P2	S
J	S2	P3	S3	S1	S1	S	S2	S1	S2	S	S1	S3	S	S2	S	S3	S	S	S2	S	S1	S	S	S5
K	S	S3	S	S	S	S	S2	S4	S4	S1	P1	S	S	S	S	S1	S	S2	S5	S	S4	S	P2	S
L	S2	P3	S3	S1	S1	S	S2	S1	S2	S	S1	S3	S	S2	S	S3	S	S	S2	S	S1	S	S	S5

● All ○ Assigned Search 77 of 77

TAC-GI Adenovirus - v1.1 (AZ Sint-Jan Brugge)

Adenovirus - H... S1 ?

Adenovirus - Bil... S2 ?

TAC-GI Astrovirus - v1.0 (AZ Sint-Jan Brugge)

Astrovirus S ?

TAC-GI Blastocystis - v1.1 (AZ Sint-Jan Brugge)

Blastocystis S ?

TAC-GI Campylobacter - v1.0 (AZ Sint-Jan Brugge)

C jcluh S1 ?

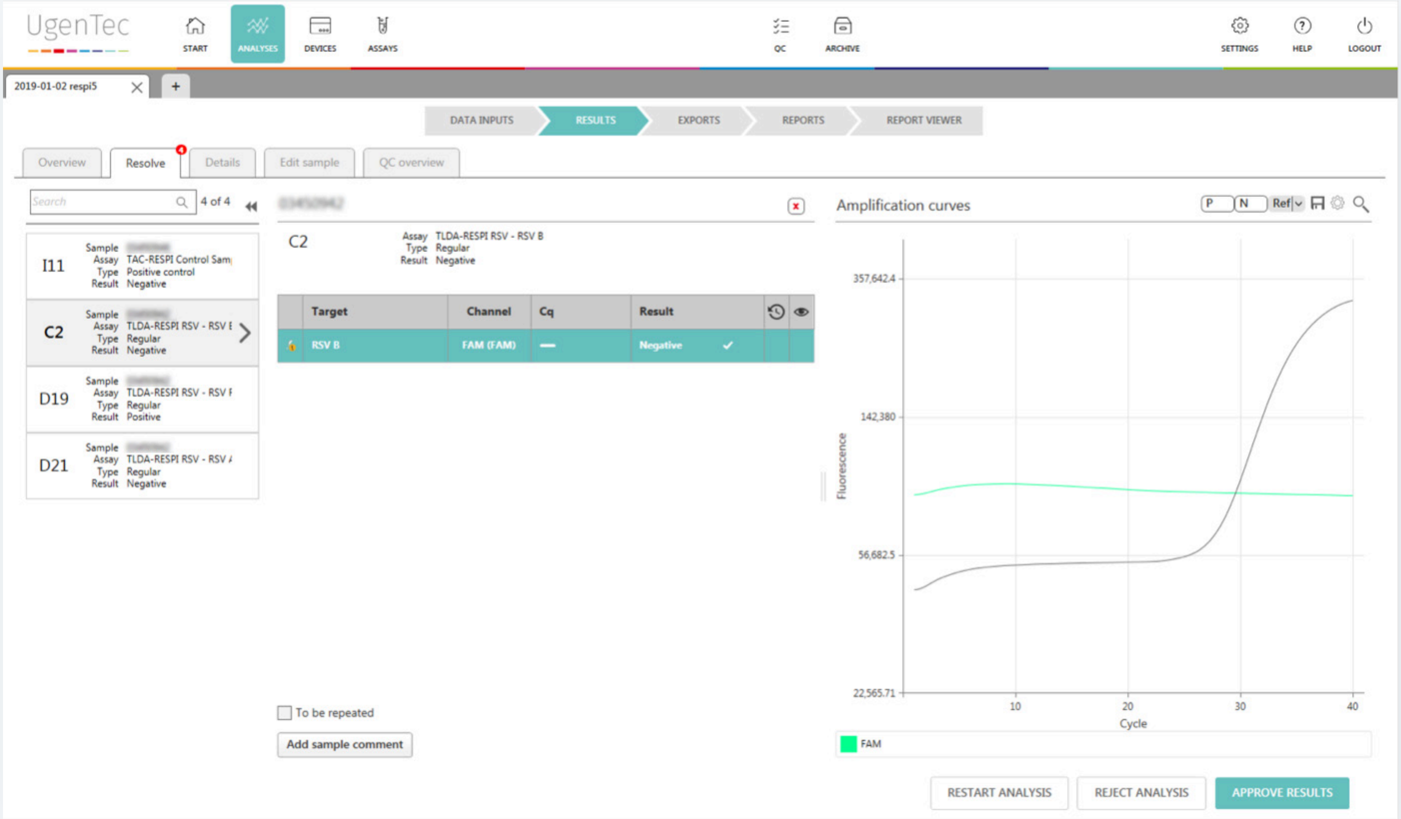
C co S2 ?

C je S3 ?

TAC-GI Clostridium difficile - v1.1 (AZ Sint-Jan Brugge)

CD A S1 ?

Fig. 3
The software uses templates to automatially detect the card layout.



I11
Sample [redacted]
Assay TAC-RESPI Control Sam
Type Positive control
Result Negative

C2
Sample [redacted]
Assay TLDA-RESPI RSV - RSV E
Type Regular
Result Negative

D19
Sample [redacted]
Assay TLDA-RESPI RSV - RSV F
Type Regular
Result Positive

D21
Sample [redacted]
Assay TLDA-RESPI RSV - RSV A
Type Regular
Result Negative

[redacted]

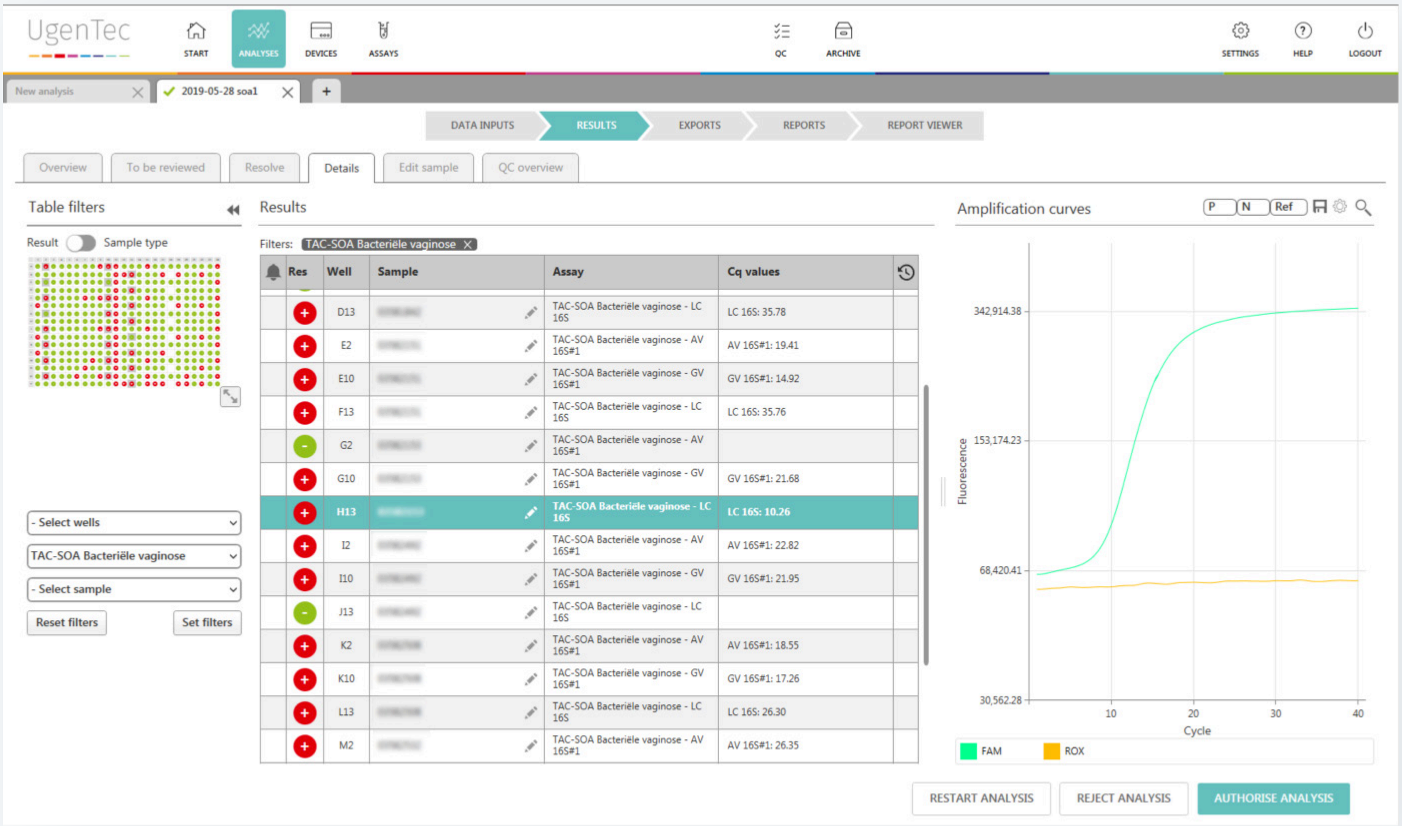
C2
Assay TLDA-RESPI RSV - RSV B
Type Regular
Result Negative

Target	Channel	Cq	Result
RSV B	FAM (FAM)	—	Negative ✓

☐ To be repeated

Add sample comment

Fig. 4
The software can be tailored to show “resolvable” samples to technicians or molecular biologists.



Filters: TAC-SOA Bacteriële vaginose

Res	Well	Sample	Assay	Cq values
+	D13	[redacted]	TAC-SOA Bacteriële vaginose - LC 16S	LC 16S: 35.78
+	E2	[redacted]	TAC-SOA Bacteriële vaginose - AV 16S#1	AV 16S#1: 19.41
+	E10	[redacted]	TAC-SOA Bacteriële vaginose - GV 16S#1	GV 16S#1: 14.92
+	F13	[redacted]	TAC-SOA Bacteriële vaginose - LC 16S	LC 16S: 35.76
-	G2	[redacted]	TAC-SOA Bacteriële vaginose - AV 16S#1	
+	G10	[redacted]	TAC-SOA Bacteriële vaginose - GV 16S#1	GV 16S#1: 21.68
+	H13	[redacted]	TAC-SOA Bacteriële vaginose - LC 16S	LC 16S: 10.26
+	I2	[redacted]	TAC-SOA Bacteriële vaginose - AV 16S#1	AV 16S#1: 22.82
+	I10	[redacted]	TAC-SOA Bacteriële vaginose - GV 16S#1	GV 16S#1: 21.95
-	J13	[redacted]	TAC-SOA Bacteriële vaginose - LC 16S	
+	K2	[redacted]	TAC-SOA Bacteriële vaginose - AV 16S#1	AV 16S#1: 18.55
+	K10	[redacted]	TAC-SOA Bacteriële vaginose - GV 16S#1	GV 16S#1: 17.26
+	L13	[redacted]	TAC-SOA Bacteriële vaginose - LC 16S	LC 16S: 26.30
+	M2	[redacted]	TAC-SOA Bacteriële vaginose - AV 16S#1	AV 16S#1: 26.35

Fig. 5
You still have access to the data generated in the details tab.

Q&A ?

What about QC, what are some of the measurements you track across the laboratory?

We maintain an ISO 15189 certified quality management system. For our cards, we run controls for each sample. These include 18S RNA controls, Phocine Distemper Virus internal control & human DNA control, ensuring sample validity and inhibition free extraction and analysis. Moreover, every new batch of array cards is thoroughly tested to ensure adherence to our quality standards.

We track these measurements for our entire laboratory in Unity Real Time by Biorad. While this software covers the essentials, it is transactional in nature. For our panels, we can track those relevant QC values in FastFinder directly and in real time, which feels a lot more integrated and automated. This is definitely a plus.

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 30,000 results retroactively. We quickly came to the conclusion that FastFinder was on par with our manual results analysis.

For our respiratory cards, across 10,368 results, performance metrics are shown below.

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

For our GI cards, performance metrics were as follows:

Parameter	Value
Balanced Accuracy	99.29%
True Positive Rate (Sensitivity)	98.58%
True Negative Rate (Specificity)	100%

Of note is that there was a small issue with the Blastocystis detection which resulted in some false negatives. This issue was consecutively resolved by the FastFinder team. This highlights that FastFinder is not just a software platform - it also comes with a team of support engineers that will work with us to resolve any issues we identify. It’s great to be able to work in a collaborative way like this.

For our STI cards, the performance metrics are as follows:

Parameter	Value
Balanced Accuracy	99.97%
True Positive Rate (Sensitivity)	99.95%
True Negative Rate (Specificity)	99.98%

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