Leveraging Automation in Analytical Analysis of Viral Vectors

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Overview

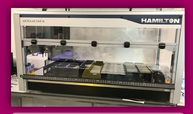
The commercial scale manufacture of lentiviral vectors (LV) presents unique problems in QC release testing. Many of the methods employed require days or weeks to complete often with multistep cell culture combined with quantitative analytical readouts. Lengthy protocols, operator-to-operator variability, and a heavy dependence on manual setup are common shortfalls. To successfully meet these challenges Oxford Biomedica (OXB) have enhanced our processes and analytics with physical and digital automation.

Our Computer-aided Biology Group design, implement and optimise lab-based robotics and digital tools to enable bench-scientists to work more efficiently, generate more robust data and reduce time-to-insight. We have leveraged automation to increase assay throughput, robustness, repeatability and significantly reduce operator hands-on-time in both Research and GxP-regulated labs. We have successfully rolled out automated systems for qPCR, ELISA / plate-based assays, mass spec sample prep and cell-culture currently performed on 12x Hamilton Microlab STARs.

In addition, our team use STARs and Gilson PIPETMAXes to perform multi-factorial Design of Experiments (DoE) to identify optima at a speed that would be impossible using manual methods only (see Poster #1356 - Rapid Optimisations for Cell & Gene Therapy Applications).

Here, we describe our learnings and the evolution of our automated qPCR workflow over 5 years running hundreds of thousands of samples.

Robotic Systems at OXB

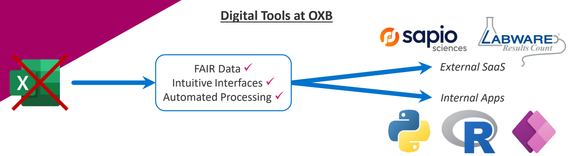


12 x Hamilton Microlab STAR

3 x Gilson PIPETMAX

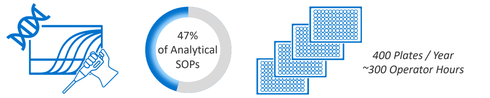
3 x Thermo QS7/Orbitor

Digital Tools at OXB



Automated qPCR – Initial Implementation

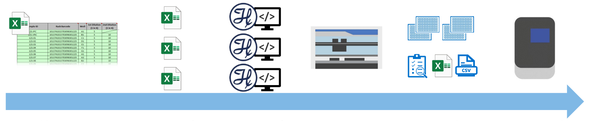
qPCR is the most widely used analytical technique for our QC release tests; both the number of different assays and the sample throughput. It was an obvious candidate for automation with liquid handlers for maximum ROI and efficiency gains.



The first iteration of our qPCR automation was designed by Hamilton; they created multiple Methods in Venus (Hamilton's programming software), each configured for a distinct SOP by hard-coding differences in standards, controls, sample processing and plate transfer.

These SOP-specific Venus Methods use an Excel-based Worklist as an input sample list to add dynamic flexibility to the Methods for each run (i.e. number of samples, target dilutions, and output plates required). Users are guided through instrument loading by a combination of Worklist-generated diagrams and interactive Venus dialogs, creating consistent and intuitive user interfaces to simplifying the transition to robotic workflows. Integrated barcode scanners verify correct loading of samples and labware and notify users of any discrepancies. Each run creates multiple output files to track the procedure, e.g. audit trail, reports, plate maps, usage metrics. Assembled qPCR plates are then loaded into a Thermo Orbitor robotic plate mover and sequentially transferred into the QuantStudio 7 thermocycler.

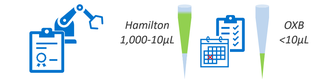
Automated qPCR Workflow



GMP Validation

Instrument Qualification

Vendor IQ/OQ; OXB PQ; regular volume verification



Computer Systems Validation

User access control; locked Venus Methods; data integrity of output files; audit trails, instrument reports & error flagging.

Method Validation

Validate VBA code in Excel Worklist generator:
- Test Venus instructions are generated correctly & invalid data gets flagged.

Validate Worklist import to Venus Methods:
- Test Worklist is read correctly & incompatible runs are detected.

Validate each SOP-specific Venus Method:
- Method Transfer to robots in lieu of full re-validation.
- Verify automated runs pass SOP acceptance criteria and show equivalence to manual assay.
- Verify on-deck stability for large batches

Automated qPCR – Continuous Improvement & Platform Maturity

After implementing the first automated qPCR methods, we focused on augmenting them with new features required for more variable Research, Process Characterisation and Assay Validation workflows.

We expanded the Excel Worklist generator to create more complex instructions for the Hamilton and adapted the original Venus Methods to create a generalised Method capable of performing any qPCR-based assay; abstracting the SOP-specific configuration from Venus into a user-configurable section in the Excel Worklist generator. This enabled lab scientists to create more dynamic assay setups via the Excel user interface (e.g. multiple assays on one plate; multiple different assay plates for one sample set; multiple totally independent plates in one run). These new options has maximised the number of assays possible on the instrument, minimised the validation burden for new assays / SOP updates, and enabled users to customise runs without learning any Venus programming.

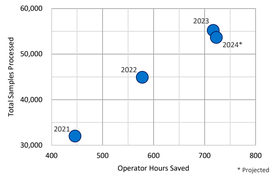
The development of python analysis pipelines allows us to combine and process output files automatically, replacing manually-completed Excel analysis spreadsheets to evaluate assay results. Summary tables automatically apply acceptance criteria and flag repeats as necessary, minimising manual assay pack QC checking.

We have also incorporated coding best-practice policies into our Venus Methods to aid long-term development of the automation systems: e.g. modularisation of code into unified shared libraries; incorporation of instrument-specific configuration files for validation-free updates; GitHub repos for Method version control & co-development.

Finally, the newest additions to our Venus Methods focus on platform robustness, looking to minimise user troubleshooting and ease potential roll out to global sites: liquid class re-optimisation; automatic tracking and seamless recovery of aborted runs; automatic webcam recording for remote assistance / troubleshooting; expanded usage metrics for in-depth troubleshooting, e.g. environmental monitoring & firmware-level logging.

Impact of Automation

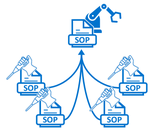
Increased Throughput & Time Savings



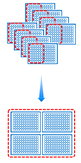
Low Sample Repeat Rate

Overnight Runs Possible

Simplified Training



Efficient Batching of Multiple Experiments



High Precision & Accuracy

	Relative Innacuracy		Precision (CV)	
Target	Automated	Manual	Automated	Manual
Duplex (Psi)	0.78%	0.29%	10.47%	11.87%
Duplex (RPPH1)	0.66%	0.88%	9.60%	13.12%
KanR	0.26%	1.60%	6.18%	11.74%
18S-Short	0.52%	0.92%	7.76%	10.58%

Supporting Automation Users

Robots break, users get confused; patience is needed. At OXB, we have created an Automation Support Hub to educate & train automation users and manage & track instrument issues & updates. Having support and information readily available to users makes them more confident to use the instruments and reduces the time spent dealing with simple issues.

Make a space to fit your needs but we have found the following useful: a public forum for contemporaneous troubleshooting, searchable as training for others; an FAQ and documentation library, easily accessible not hidden in SOPs; centralised logging of errors to identify patterns & bugs more easily; continuous improvement suggestions from users to better target updates; on-call rota for automation experts to triage problems and minimise disruption to the whole automation team.

Advice for Automation Adopters

Plan Long-Term
- Quick implementation = prolonged optimisation.
- Be thorough up-front e.g. precise liquid classes & labware definitions.
- Log everything! Structure and store all metadata your instrument can produce.
- Design methods to allow later additions. There will always be a new feature request.
- Generalise robot Methods and abstract coding to less niche languages. ELN for user input; Python for logic, calculations & data validation; Venus for pipetting and transport only.
- Pick the right tool for the job: recent rise of entry-level automation options make small multi-instrument workcells a viable alternative to traditional large liquid handlers. Consider miniaturisation options.

Identify Key Drivers & Plan for Future Drivers
- Automation can be tailored for many benefits but not all at once.
- Are you optimising for speed, throughput, accuracy, hands-off-time…?

Bring Skill Set In-House
- Automation is a continuous project; it is not a once-and-done transformation.
- Building an automation applications team in-house allows faster Method deployment & development due to a better understanding of your science; better adoption and support for lab users (training and troubleshooting); less instrument down-time & more recovered assays; maximise instrument usage by identifying new workflow synergies.
- Engage in automation user groups to share experiences.

Support Adoption
- It is essential to communicate with automation users and set in-depth URS. De-mystify automation and manage expectations. Listen and take feedback on-board.
- Identify automation champions to advocate benefits to their groups.
- Convince users with features. Convince scientists with data. Convince managers with metrics.
- Be sympathetic to technophobes and traditionalists…

Links

Webinar: Automation for GMP Vector Production

Case Study: Automated Cell Culture - Vector Titre Assay

Webinar: Automated Analytics for Viral Vectors

Contact: Oxford Biomedica partnering@oxb.com

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