Automating Cell-Based Assays to Determine Viral Vector Titer

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Summary

Oxford Biomedica is an innovative leading viral vector specialist focused on delivering life-changing therapies to patients. The Computer-Aided Biology (CAB) Group is part of Oxford Biomedica's Platform Research department and is responsible for developing, qualifying, and validating key analytical assays related to the final therapeutic compound. In this Case Study, we focus on the integration assay, an analytical assay used to determine viral vector titer. We describe in detail the automation of the 10-day cell-based workflow preceding the quantitative Polymerase Chain Reaction (qPCR)-based assessment of the stable integration of therapeutic viral vector sequences.

About Oxford Biomedica

Oxford Biomedica is a global gene and cell therapy company headquartered in Oxford, United Kingdom (UK). The company works across key viral vector delivery systems (lentiviral-, adeno-associated viral- and adenoviral-based vector systems), providing innovative solutions to cell and gene therapy biotechnology and biopharma companies for their process development, analytical development and manufacturing needs. Dr. Kyriacos Mitrophanous, Chief Scientific Officer at Oxford Biomedica, commented: "Gene and cell therapies are the most innovative modalities of therapeutics in development at the moment. These therapies are seeking to address the underlying causes of disease, and the aim is to achieve a one-off treatment that is lifelong."

Oxford Biomedica has state-of-the-art facilities in the UK and United States (US), and more than 900 employees worldwide. In Oxfordshire, UK, there are more than 200,000 sq. ft. of manufacturing, laboratory and office space spanning six facilities, including seven Good Manufacturing Practice (GMP) suites operating next-generation single-use 200L and 1000L bioreactors, as well as two fill/finish suites.

Oxford Biomedica has built a sector-leading lentiviral vector delivery system, the LentiVector® platform, and is working on programs from pre-clinical to commercial stages across a range of therapeutic areas with global partners. In 2022, the company established Oxford Biomedica Solutions, a US-based subsidiary specializing in Adeno-Associated Virus (AAV).

Learn more about Oxford Biomedica

Analytical Assays at Oxford Biomedica

The Analytics groups at Oxford Biomedica offer an exceptionally comprehensive suite of in-house assays, ensuring full lentiviral vector characterization, Quality Control (QC) and stability testing, and preparing Chemistry, Manufacturing and Controls (CMC) components for regulatory filings. The analytical assays at Oxford Biomedica are carried out by four groups in charge of different parts of the research and clinical manufacturing process. The Platform Research

Group (PRG), in particular, is responsible for developing, qualifying, and validating key analytical assays related to the final therapeutic compound. Dr. André Raposo, Director of Analytics, Platform Research, Computer-Aided Biology Group at Oxford Biomedica, explains: "In PRG, we leverage technologies such as artificial intelligence/machine learning, Design of Experiments (DOE) services and liquid handling automation to develop and support most analytical assays. These assays later transition into the Analytical Service Group to be used during GMP QC, before batch release."

Liquid Handling Automation of Analytical Assays

The Analytics groups at Oxford Biomedica currently perform automated workflows using Hamilton liquid handling systems for four main types of assays: cell culture, qPCR setup, Enzyme-Linked Immunosorbent Assay (ELISA) and sample preparation for mass spectrometry. In total, the company has 12 Hamilton Microlab® STAR™ line platforms in GMP and others in research. Three of the systems are used for the integration assay, two in Research and Development (R&D) and one in GMP (**Table I**).

Dr. Raposo commented: "We were initially performing the integration assays manually; however, we were faced with several challenges due to long experiment times, complex workflows, heavy dependence on manual work and considerable paperwork. We decided to automate the assay using Hamilton systems due to their flexibility. The company already had multiple Hamilton systems in-house, and there was a very good knowledge of Hamilton's VENUS software which was important for troubleshooting during the development stage. Moreover, we knew that the Service and Application teams offered quick and high-level support."

Automated Workflows for the Assessment of Viral Integration

One of the most complex analytical assays is the integration assay. The integration assay is essential to measure the efficacy of integrating lentiviral vectors by determining their ability to insert genetic material into target cells. The complete assay involves transducing human cells with the viral vectors at various concentrations, passaging cells to dilute out unintegrated DNA species, and quantifying the number of packaging signals incorporated into the cells' genomes via qPCR.

System Overview

The system for the integration assay is based on a Hamilton Microlab® STAR™ with eight 1000 µl pipetting channels, CO-RE® Grippers and an iSWAP® Arm, enclosed on a class II cabinet from Contained Air Solutions. The automated liquid handler is integrated with a Thermo Fisher Scientific™ Cytomat™ 5C Automated Incubator and a Thermo Fisher Scientific™ Cytomat™ 10 Hotel Ambient Storage, which are connected to the Microlab® STAR™ deck via two linear tracks (**Figure 1**). The deck includes various modules, including a Liquid Dispenser, a Temperature-Controlled Carrier and two Tilt Modules (**Figure 2**).

Mr. Karim Benyaa, an International Project Leader at Hamilton Robotics, explains the advantage of the system's configuration: "Dr. Raposo's Team was looking for a cell culture system that would allow them to process a large number of samples with minimal user intervention and could run several different experiments simultaneously over long periods. The solution we offered was to integrate the Microlab® STAR™ with a plate hotel and an incubator so

Table I: *Automated Hamilton Systems at Oxford Biomedica*

System No.	Assay Type	Assay Aim	Regulation
1	Replication-Competent Lentivirus Co-Culture (RCL-CC) – Cell-based assay	Detection of Replication-Competent Lentivirus by co-culture in Contaminant Level 3 (CL3)	GMP
2			GMP
3	Integration assay – Cell-based assay	Determination of lentiviral vector titer	R&D
4			
5			GMP
6	PCR	Quantification of multiple targets (e.g., lentiviral vector titer residual DNA)	R&D
7			
8			GMP
9	ELISA sample dilutions, DOE supported by Synthace R&D Cloud platform	Media optimizations, lentiviral vector transfection optimizations, determination of p24 vector capsids	R&D
10	ELISA sample dilution	Determination of p24 vector capsids, benzonase, Host Cell Protein (HCP)	R&D
11			GMP (in preparation)
12	Mass Spectrometry sample preparation	Proteome analysis	R&D

Figure 1. System Overview. *(1) Cytomat™ 5 C Automated Incubator, (2) Hamilton Microlab® STAR™, (3) Linear Track, (4) Cytomat™ 10 Hotel, (5) Contained Air Solutions Class II Cabinet.*

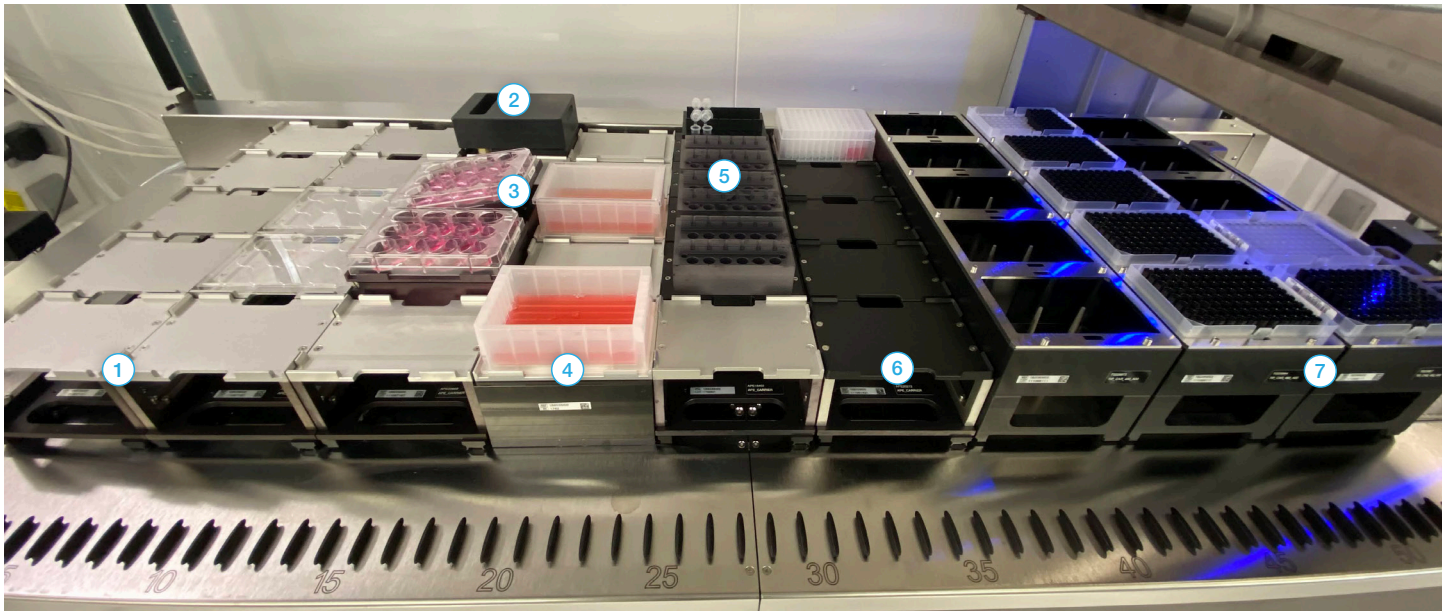


Figure 2: Deck Layout of Customized Hamilton Microlab® STAR™. *(1) 2X carriers for microtiter plates/lids, (2) Liquid Dispenser (used as a waste module), (3) 2X Tilt Modules with plates containing the cells to be passaged, (4) Temperature-Controlled Carrier with reagents (in troughs), (5) carrier with adaptors for Eppendorf Tubes®, (6) carrier for DWPs, (7) 3X tip carriers.*

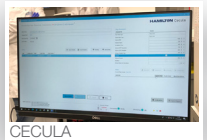
that the full method could be controlled by Hamilton VENUS software and CECULA's Graphical User Interface (GUI). This configuration not only allows for a walk-away solution but also ensures the traceability of all samples and reagents. Furthermore, enclosing the liquid handler in a class II cabinet ensures sterility of this long and complex workflow, including the dispensing and loading of reagents."

Description of the Workflow

The workflow is divided into three automated methods (**Figure 3**):

1. Cell seeding (Day -1)
2. Vector dilution and inoculation (Day 0)
3. Cell passaging and harvesting (Days 3-10)

All steps are performed in 12-well plates, except for the harvest, which is done in 96 Deep-Well Plates (DWPs).


CECULA

Plan the steps of the workflow in CECULA

Check the resources for the steps to be run on that day in CECULA

Load labware in Plate Hotel

Cell Seeding
Day -1

HEK293T cells in suspension are loaded on the deck of the Microlab® STAR™

Cells are seeded in 12-well plates

Plates with cells are moved to the incubator

Vector Dilution & Inoculation
Day 0

Viral vectors and media are loaded on the deck of the Microlab® STAR™

Viral vectors are diluted

Plates with cells are brought from the incubator to the deck

Cells are inoculated with viral vectors

Plates with cells are moved to the incubator

Cells are incubated for three hours

Plates with cells are brought from the incubator to the deck

Fresh media is added to the cells

Plates with cells are moved to the incubator

Cell Split and Harvest
Days 3, 5/6, 7/8

Media, TrypLE® and PBS are loaded on the deck of the Microlab® STAR™

Plates with cells are brought from the incubator to the deck

Plates are tilted and the media is removed

PBS is added and plates are tilted back-and-forth

Plates are tilted and the PBS is removed

TrypLE® is added and plates are tilted back-and-forth

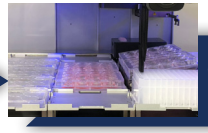
Plates are incubated in a flat position for 5 min

Fresh media is added and cells are homogenized

if required

A defined volume of each well is transferred into a new (daughter plate) with fresh media

Plates with cells are moved to the incubator



Cell Harvest
Day 10

A sample volume is transferred to a 96 DWP for downstream processing

Manual Task

Automated Task performed on integrated third-party devices

Task performed on Hamilton automated systems

Figure 3: Automated Workflow for the Viral Vector Integration Assay.
DWP: Deep-Well Plate; PBS: Phosphate Buffered Saline

The key feature of the automation of this workflow is planning. Dr. Raposo explains, "We plan the execution of each workflow step using CECULA's GUI. In CECULA, we input which steps should be executed for each batch and when. Once the initial setup is performed, the system is fully autonomous and able to process all queued plates on a particular batch with no operator intervention".

Before beginning any method, all reagents must be loaded onto the deck (e.g., media, TrypLE™, Phosphate Buffered Saline (PBS)). Empty barcoded labware (12-well plates and 96-DWP) is introduced to the system via the plate hotel to ensure full traceability of all plates across the ten-day workflow. CECULA informs the user about the consumption of resources, letting them know about any upcoming shortages.

Mr. Benyaa commented on CECULA features "CECULA is a user-friendly GUI running on top of Hamilton's VENUS software. It is designed for customers running cell-based assays of multiple batches over several days. What makes CECULA so useful for these types of assays is that it is easy to use while at the same time allowing a lot of flexibility to plan long-term and complex workflows."

The first step of the automated workflow is seeding human cells into 12-well plates. For this, a trough of HEK293T cells is manually loaded into the system for automated seeding on 12-well plates, mixed well, and dispensed into the plates in pairs. After seeding, the plates containing the seeded cells are automatically placed inside the incubator. Dr. Raposo highlighted, "Once seeded, the cells are always stored in the incubator and only brought onto the deck when a new step is performed. After the method is completed, the cells are again automatically placed inside the incubator. This ensures that the cells remain healthy and viable".

The day after the seeding, the vials containing the viral vectors to be assessed are loaded onto the deck in Eppendorf Tubes®. The Microlab® STAR™ then performs the desired vector dilution and subsequent inoculation on the cells. For cell passaging and harvest, the plates containing the cells and liquid reagents are placed on the Tilt Modules and the Temperature-Controlled Carrier on the Microlab® STAR™, respectively. The protocol involves (i) removal of old media, (ii) a wash with PBS, (iii) addition of TrypLE™ for cell detachment, (iv) 5 min incubation with up/down tilt cycles every 30 s, and (v) addition of fresh media and single cell homogenization.

Mr. Benyaa commented on the use of the deck modules for these steps, "The Tilt Module mimics the manual tilting of the plate, facilitating the removal of liquid from the wells and the even distribution of the reagent. Furthermore, the Temperature-Controlled Carrier maintains the media and the TrypLE™ at 37°C, ensuring the optimal conditions for cell detachment and viability."

Once the cells are fully detached, a pre-determined volume of each well is transferred to a new (daughter plate) with fresh media. In addition, and if required as per protocol, a chosen volume of each well is transferred to a 96-DWP for further processing (i.e., DNA extraction and PCR or flow cytometry). On day 10, cells are not split further, and only the sample for further processing is taken.

Dr. Raposo commented on the handling of the liquid on- and off-deck, "The on-deck Liquid Dispenser is a versatile tool within the system. It is used to drain on-deck old media into an off-deck waste bottle containing disinfectant and pumps off-deck PBS onto the deck to rinse tips before disposal."

Downstream sample processing is performed using a QIAGEN QIAcube HT for nucleic acid extraction, a (different) Microlab® STAR™ for qPCR setup, and an Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System for qPCR. The qPCR primers and probes are designed to target a viral sequence and a human housekeeping gene.

Benefits of the Automated System

Software – Ease and Flexibility

The automated cell-based workflow for the assessment of lentiviral vector titer was developed jointly by Oxford Biomedica and Hamilton Robotics. The implementation of the methods took approximately eight months from system arrival to routine sample runs. Dr. Raposo commented, "We have established a productive collaboration with Hamilton's Application Team. Hamilton developed the original methods in simulation to meet our User Requirement Specifications (URS). Then, as part of the Site Acceptance Testing (SAT), the methods were tested using water runs. This is a routine procedure when implementing automated workflows using liquid handlers. After this, we performed further optimization using biological samples at Oxford Biomedica. Over the course of implementation, my team has become proficient in Hamilton's VENUS Software and CECULA. CECULA, in particular, is user-friendly, intuitive and flexible. We can easily modify the volumes in a method or the number of samples without having to touch the underlying code."

Throughput and Walk-Away Time

With the current setup, the platform is able to process more than one thousand 12-well plates in a year without the need for after-hours or overnight operations. Dr. Raposo commented, "We currently do not require the system's full capacity and typically run batches of seven 12-well plates per day. However, we are equipped to increase our current throughput whenever needed."

When performed manually, a standard 10-day workflow of a batch (seven 12-well plates) typically requires 19 hours of operator time (hands-on time) divided between two operators. In comparison, the automated method only requires 3.25 hours of operator time (**Table II**). Dr. Raposo commented, "The automation of this method reduces operator time to less than a fifth of the time it would take manually. This is a remarkable advantage since it frees up our operators' time and allows them to work on other activities while the system is running."

Table II: Comparison of the Operator Time (hands-on time) Required for the Manual vs. Automated Method.

7 x 12-well plate batch, over 10 days (5 working days)

Step	Operator Time (Automated)	Operator Time (Manual)	
		Operator 1	Operator 2
Transduction	1.25 h	3.5 h	3.5 h
P1	0.5 h	1 h	1 h
P2	0.5 h	1 h	1 h
P3	0.5 h	1 h	1 h
Harvest	0.5 h	3 h	3 h
Total	**3.25 hours**	**9.5 x 2 = 19 hours**	

<0.5 Man-Days and 1 Operator vs. **~2.4 Man-Days and 2 Operators (1.2 Man-Days for 1 Operator)**

Compliance with GMP requirements

The automated cell-based workflow for the assessment of lentiviral vector titer has now been transferred to the Analytical Service Group to be used in GMP QC for batch release. We now have separate instruments based on compliance – R&D versus GMP. Dr. Raposo commented: "To transfer such complex scripts and workflows from R&D to GMP, we had to adapt the system to comply with the Federal Drug Administration (FDA)'s 21 CRF Part 11 regulations. For this, we worked with Hamilton's Application Team to implement and validate compliance aspects, such as user access restriction, data integrity, full sample traceability and time-stamped audit trails, to name a few features." Mr. Benyaa explained how one of these requirements, the user access restriction, was set up: "We developed a special user authentication function for CECULA, which is connected to the Windows user accounts. With this new function, we are able to define groups with specific permissions to handle CECULA. For example, one group is allowed to monitor the CECULA software and refill resources but cannot create or change batches. This setup also allows us to trace who is doing what and when."

As part of the assay transfer into GMP, a series of studies was performed to assess the comparability of the manual workflow versus the automated assay. A summary of the results is shown in **Figure 4**.

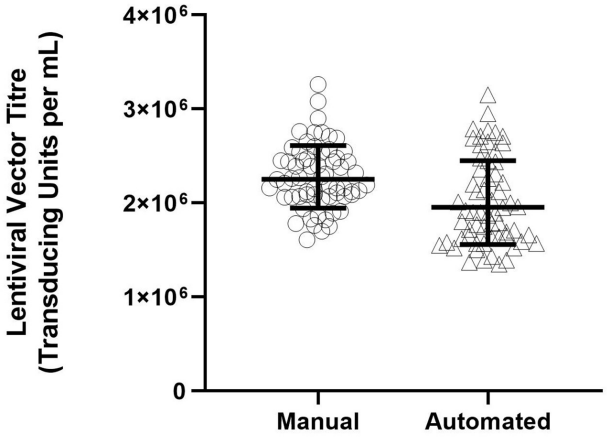


Figure 4: Summary of comparability studies. *Three independent assays were set up and fully conducted manually by three different operators (Manual) or on the Automated system (Automated). HEK293T cells were transduced in triplicate wells with seven different lentiviral vector reference control dilutions in 12-well plates. After ten days, cells from both assays were harvested and submitted to DNA extraction and qPCR. Scatter-dot plots represent multiple replicates. Lines depict geometric means with geometric Standard Deviation (SD) of lentiviral vector titer in transducing units per mL (TU/mL).*

"The development, optimization and implementation of the automated cell-based assay for the determination of lentiviral vector titer has been a major achievement for Oxford Biomedica. This was possible through the dedication and forward thinking of the different analytical departments at Oxford Biomedica with the collaboration of the technical team at Hamilton. The automation of this particular assay has allowed us to increase throughput while freeing up operators to do less repetitive tasks. Oxford Biomedica is currently working on expanding and transitioning more automated methods from R&D to GMP using Hamilton platforms," said Dr. Raposo.

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