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Analytical Methods for Viral Vector Development and Manufacturing in **Gene Therapy**

'The Eleventh eBook in the Hamilton series'

Foreword

Gene therapy is one of the most innovative therapies in the world. By Q4 2022, there were 24 gene therapies approved and more than 2,000 clinical trials for gene therapies being conducted worldwide (including genetically modified cell therapies).[1] Although there are several methods to transfer genetic material into cells, viral vectors are the most popular ones, particularly those based on Adeno-Associated Viruses (AAVs), retroviruses, lentiviruses and adenoviruses.

As with any other therapeutic product, the development and manufacturing of viral vectors for gene therapy must follow Good Practice (GxP) regulations. A Good Manufacturing Practice (GMP)-compliant process for viral vectors must ensure consistent quality in the vector's potency (biological activity), purity (absence of contaminants or impurities) and safety (absence of potentially harmful substances). To achieve this, various "Critical Quality Attributes (CQAs)" are monitored during the manufacturing process by measuring key properties and ensuring that they are within the appropriate levels.[2] Quick, reliable and high-throughput analytical methods to assess these CQAs are essential in process development and later GMP-compliant testing for batch release during Quality Control (QC). Given that automated liquid handlers dramatically improve results' reproducibility and samples' traceability, they are inherently suitable for high regulatory compliance.

In this eBook, we review the history of gene therapy, discuss the regulatory and technical requirements of the analytical methods used to develop and manufacture viral vectors, and describe how Hamilton's automation solutions can fulfill them.

This eBook is part of a dedicated campaign on Analytical Methods for Viral Vector Development and Manufacturing in Gene Therapy. Our scientific campaigns aim to provide our readers with interesting educational resources and a close-up view of how customers use our solutions to accomplish their tasks.

I want to thank AGC Biologics Milan and Oxford Biomedica for their valuable insights into these topics.

We hope you find the content beneficial.

Your kind feedback is always highly appreciated.

Yours sincerely,
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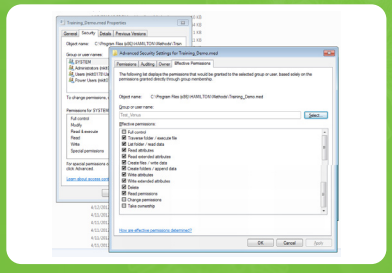

Hamilton Software

Hamilton's Automated Solutions
For ELISA, qPCR and Cell Culture

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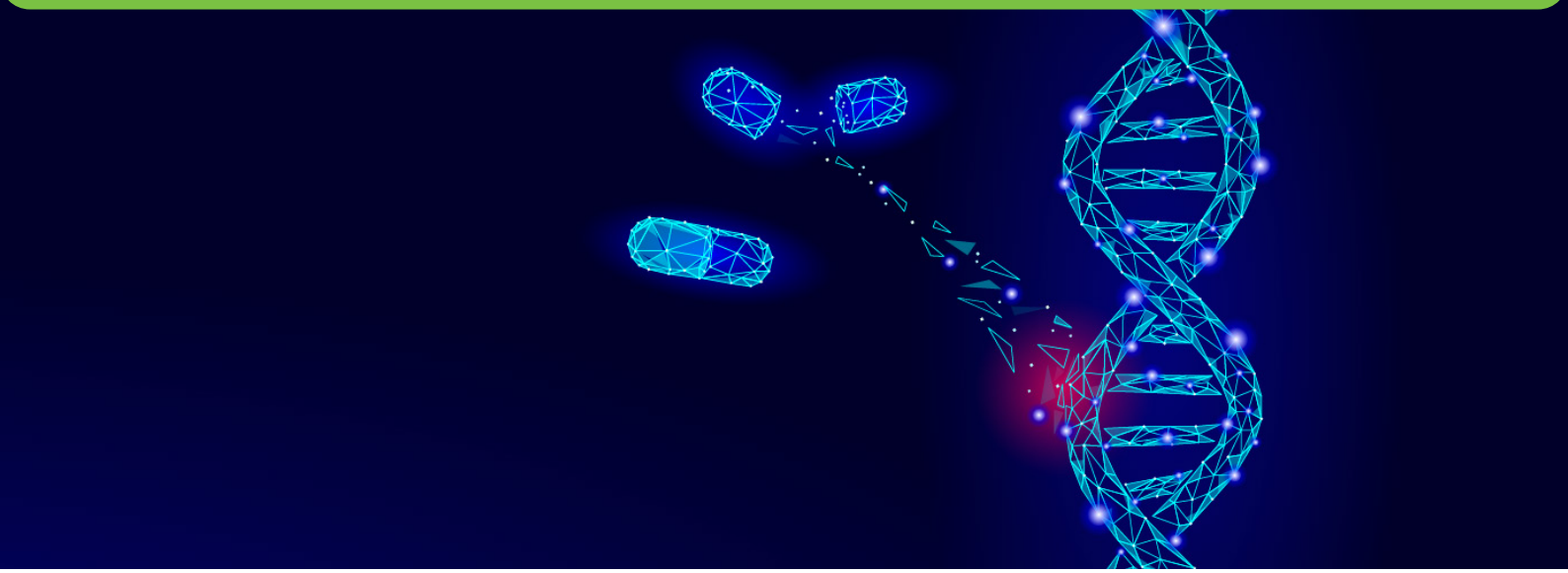

The Emergence of a Complex Field: Gene Therapy

A Brief History of Gene Therapy

Gene Therapy is defined as "the treatment of disorder or disease through the transfer of engineered genetic material into human cells".[1] Unlike messenger Ribonucleic Acid (mRNA) or viral-based vaccines, gene therapies make deliberate changes to the DNA of the target cells by (i) adding a functional copy of a gene, (ii) turning off or (iii) replacing a gene that produces a defective molecule or by (iv) changing the regulation of a gene.[2,3] Although several methods exist to transfer genetic material into cells (e.g., RNAs, plasmids, oligonucleotides), viral vectors are the most popular.[4]

There are two current approaches for gene therapy: *in vivo* and *ex vivo*. *In vivo* gene therapies deliver genetic material directly into the patient, commonly through injections. *Ex vivo* gene therapies, on the other hand, involve the extraction of the patient's cells, the *in vitro* delivery of the genetic material, and the introduction of the modified cells back into the patient's body.[5]

The first approved gene therapy trial took place in 1990 in the United States. Researchers at the National Institutes of Health (NIH) treated a four-year-old girl with Severe Combined Immunodeficiency (SCID) using an *ex vivo* strategy. More specifically, they added a functional copy of a gene coding for the missing enzyme responsible for the disease (Adenosine Deaminase or ADA) into her white blood cells, using a retroviral vector.[6] Since then, gene therapy has gradually gained importance as a medical approach due to its potential to provide lasting therapies or even cure diseases previously considered incurable by transferring therapeutic nucleic acids (DNA or RNA) into a patient's cells or correcting defective genes via gene editing.[7]

The path of this innovative therapy has, however, not been straightforward. Several cases of insertional mutagenesis leading to leukemia and immune reactions slowed down the progression of the field between 1999 and 2002.[4,8] One of the most memorable setbacks in the field occurred in 1999 when an 18-year-old patient died during a clinical trial, due to massive organ failure triggered by an immune reaction to the viral vector used.[9]

As a result of these setbacks, several genes were removed from the viral vectors to make them safer, and new guidance/regulations to ensure product safety and efficacy were issued.[10] In the western world, the most important gene therapy guidelines include those published by the United States Food and Drug Administration (FDA) and the Office of Tissues and Advanced Therapies (OTAT)[11] and Europe's European Medicines Agency (EMA), the Committee for Advanced Therapeutics (CAT)[12], and the International Conference of Harmonization (ICH) (S12[13,14]). For more detailed information about regulatory considerations for gene therapy products, see Halioua-Haubold 2017.

In 2012, after many improvements in the field, the first gene therapy was approved for commercialization in the western world by the EMA: Glybera, intended to treat Lipoprotein Lipase Deficiency.[15,16] Unfortunately, Glybera was later withdrawn from the market due to its high price (~ USD $1 million per treatment) and failure to obtain national reimbursement in Europe.[17] The price of gene therapies has been and continues to be a matter of controversy, especially because these treatments offer a very high cost-benefit ratio in some or all patients, and current payment models are not suitable for these types of therapies.[17]

Despite these controversies, the gene therapy field has kept growing, and several other products have successfully come onto the market, including Strimvelis (to treat SCID), Luxturna (to treat retinal dystrophy), Zolgensma (to treat Spinal Muscular Atrophy), Kymriah (to treat blood cancer) and Libmeldy (to treat metachromatic leukodystrophy).[4,18,19,20,21,22] Five new gene therapies were approved in the year 2022, which contributed to increasing the total number of approved gene therapies globally to 24.[23] Furthermore, more than 2,000 clinical trials are currently being conducted worldwide, emphasizing the field's vast potential.[23,24] It must be highlighted that the discovery and evolution of precise gene-editing tools such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have gained importance in the field and are contributing to its growth. Successful gene therapy clinical trials utilizing these new gene-editing systems have already been reported.[25,26]

Due to the fact that *ex vivo* gene therapy involves the transfer of modified cells into the patient, this approach is tightly linked to cell therapy.[27] In this eBook, however, we will not be discussing the aspects specific to cell therapy and will focus on viral vectors as vehicles for the delivery of genetic material.

Viral Vectors for Gene Therapy

As previously described, viral vectors are the most common vehicle for the transfer of genetic material. The four most common types are based on Lentiviruses (LVs), Adeno-Associated Viruses (AAVs), retroviruses and adenoviruses. However, the first two are currently preferred as they are more efficient at gene delivery and have a lower risk profile than retrovirus-based vectors.[7, 28]

AAVs are highly effective for *in vivo* gene delivery due to their diverse capsids and serotypes, which can thus target a wide range of tissues and cell types. Furthermore, they do not cause any known illness in humans and have a low risk of insertional mutagenesis. On the other hand, retroviral and lentiviral vectors are best suited for *ex vivo* gene therapies where the integration of the therapeutic gene into the patient's cell is desired and can be carefully monitored. This stable integration provides lasting effects.[4,7]

In general, the production of viral vectors requires six main steps: (i) culture and expansion of packaging cell line, (ii) plasmid transfection, (iii) clarification (e.g., through centrifugation or filtration), (iv) purification (e.g., through filtration or chromatography), (v) concentration (e.g., through ultracentrifugation) and (vi) fill & finish (or packaging).[29] Various analytical assays are performed throughout this process to ensure the quality of the process and final product.

The Role of CROs, CMOs and CDMOs

The success of a gene therapy product depends not only on the induction of the expected biological response but also on the effective navigation of the challenges associated with developing, manufacturing, and delivering these complex products. In general, there are five main steps to any drug development process: (i) discovery and development, (ii) clinical manufacturing (pre-clinical and clinical trials), (iii) regulatory body review and approval (FDA in the US and EMA in the European Union), (iv) commercial manufacturing, and v) post-market surveillance.[30]

Each of these five steps entails several challenges and considerable amounts of resources and time. Developing a gene therapy can cost up to USD $5 billion, and clinical trials can take eight years or more.[31, 32] Due to these challenges, biotechnology and pharmaceutical companies often rely on third-party partners with expertise in technical, manufacturing, and regulatory areas. Contract Research Organizations (CROs), Contract Manufacturing Organizations (CMOs) and Contract Development and Manufacturing Organizations (CDMOs) like Lonza, Catalent, Pantheon, Oxford Biomedica and AGC Biologics provide a wide range of services, including discovery research, product development, clinical trial support, manufacturing, and commercial supply of the final product.[33]

CROs typically provide support during early discovery research, CMOs offer manufacturing expertise and infrastructure, and CDMOs offer more comprehensive services ranging from formulation development to commercial manufacturing.[34] There is, however, an increasing degree of overlap between these organizations.[35]

Regarding product development and manufacturing, the collaboration with a CDMO might begin as early as the pre-clinical stage. As part of the formulation design, the CDMO may create Good Manufacturing Practices (GMP) and non-GMP prototypes for pre-clinical and clinical trials (i.e., clinical manufacturing) and actively participate in their redesign. In addition to developing the necessary analytical methods to ensure strict Quality Control (QC), CDMOs maintain meticulous records of all relevant documentation, results, and administrative data (e.g., FDA 21 CFR Part 11 compliance). They provide assistance throughout the submission of clinical trial results, pre-clinical testing information, manufacturing, QC, and administrative information to a regulatory body for assessment.[36] If the regulatory agency approves the therapy for commercialization, the process of scaling up to commercial manufacture begins (i.e., bulk production, packaging, and supply chain processes). CMOs, similar to CDMOs, support the manufacturing processes from prototype production to commercial manufacturing; however, they are not actively involved in formulation development.

There is a significant need for CMOs and CDMOs in the gene therapy industry due to the intricacy of the manufacturing processes and products. The following sections will discuss critical parameters to assess when developing and manufacturing gene therapy products.

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VIRAL VECTOR DEVELOPMENT AND MANUFACTURING FOR GENE THERAPY

CDMO

CRO

CMO

DISCOVERY

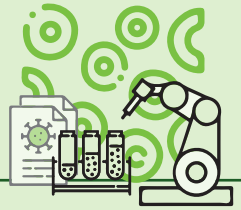
Research Lab

Target validation, profiling and cell engineering

CLINICAL PHASE

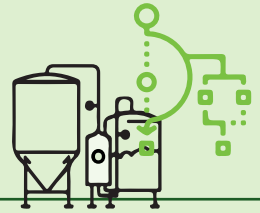
Development Lab

Formulation design, GMP and non-GMP prototypes



Clinical Manufacturing

Production of the viral vector for Clinical Trials



Clinical Trials

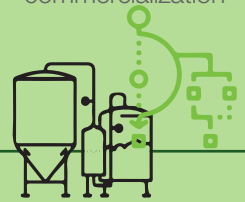
Phases 1 to 3 and active formulation redesign

REGULATORY APPROVAL OF THE THERAPEUTIC PRODUCT

COMMERCIAL PHASE

Commercial Manufacturing

Production of the viral vector for commercialization

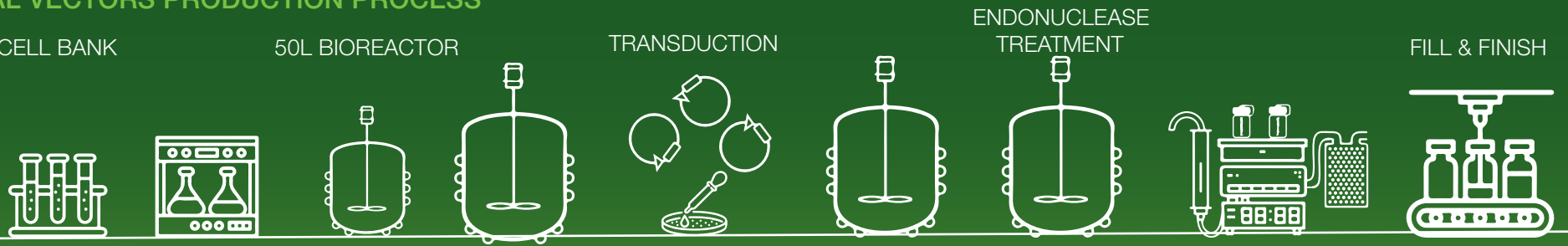


Supply Chain

Warehouse storage and temperature-chain maintenance

Analytical Development and Quality Control

VIRAL VECTORS PRODUCTION PROCESS



UPSTREAM PROCESSING

DOWNSTREAM PROCESSING

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Why Do We Need Automation for Viral Vector Development and Manufacturing?

Good Practice (GxP) - compliance

Viral vector manufacturing presents unique challenges compared to the manufacturing of small compounds or conventional biopharmaceuticals. Some well-known challenges include the inherent variability of the starting material (e.g., plasmids, producer cell line), lack of reference standards, risk of insertional mutagenesis (for integrating vectors), use of active ingredients and multiple sources of contamination. All of these parameters directly influence the safety and efficacy profile of the final therapeutic product, including aspects such as toxicity, immunogenicity, oncogenicity and environmental risk due to shedding.[1,2]

As with any other therapeutic product, the development and manufacturing of viral vectors for gene therapy must follow Good Manufacturing Practices (GMP) during clinical and commercial product manufacturing. GMP regulations apply to every step of the manufacturing process: people, premises, processes, products, and procedures.[3-5]

A GMP-compliant manufacturing process for viral vectors must ensure consistent quality in the viral vector's potency (biological activity), purity (absence of contaminants or impurities) and safety (absence of potentially harmful substances). To achieve this, various "Critical Quality Attributes" (CQAs) are controlled during the manufacturing process by measuring key physical, chemical, biological and microbiological properties and guaranteeing that they are within an appropriate limit, range or distribution".[5] Quick, reliable and high-throughput analytical methods to assess these CQAs are essential in process development and later GMP-compliant testing for batch release during Quality Control (QC). Since the regulatory requirements are higher in QC (GMP) than in Research and Development (R&D), the analytical approaches designed during product development must be re-evaluated, updated, and validated before transfer.

Given that most automated liquid handlers dramatically improve results' reproducibility and samples' traceability (including the integration into the Laboratory Information Management System (LIMS)), they are inherently suitable for high regulatory compliance. Furthermore, some of these automated systems are equipped with all the features necessary to transfer automated methods developed in R&D (i.e., process development) to GMP-compliant QC testing.

Analytical Methods – common techniques during viral vector manufacturing

The most common analytical methods to assess viral identity, yield, potency, purity and safety during manufacturing rely on variations of two main techniques: Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). Other analytical methods include anion exchange chromatography, flow cytometry, capillary electrophoresis, dynamic light scattering, and electron microscopy.[5,6]

Despite its simplicity, ELISA procedures contain numerous repetitive steps with lengthy incubation times, making them

prone to manual errors. Complete ELISA assays (from standard dilution to plate reading and results reporting) can be automated by integrating on-deck or peripheral devices into automated liquid handlers. Moreover, automation facilitates the parallel run of various assays using the same sample aliquot, increasing overall throughput and economizing precious product samples.[7]

PCR-based analytical methods require complicated workflows that, when performed manually, are highly fragmented (e.g., DNA/RNA extraction, DNA/RNA quality control, PCR set-up, amplification, and detection), increasing the likelihood of errors and difficulties with the traceability of the samples. Similar to the automation of ELISA workflows, the automation of the PCR workflows can be done for single steps or the complete workflow: "sample-to-results" solutions. We have previously discussed the advantages of automating PCR workflows in our eBook: Molecular Diagnosis of Infectious Diseases.

In addition to the automation of end-point assessment techniques (i.e., ELISA and PCR), automated liquid handlers can also support preparatory assays run before the final assessment, including those requiring the culture and handling of cells. Two of the most common ones are (i) infectious viral titer, a potency assay that measures the biological function of the viral vector and (ii) Replication Competent Virus (RCV), a safety assay that assesses the inability of the viral vector to replicate. Automated liquid handlers offer significant advantages for handling cell cultures, thanks to the sophisticated technologies and dedicated software available in a handful of the latest-generation workstations. These automated systems can ensure precise and gentle aspirating and dispensing, controlled temperature and shaking, and sterility, as well as allow for the integration of third-party devices for automated analysis. We have previously discussed the advantages of automating cell-based assays in our eBook: Cell-Based High-Throughput Screening.

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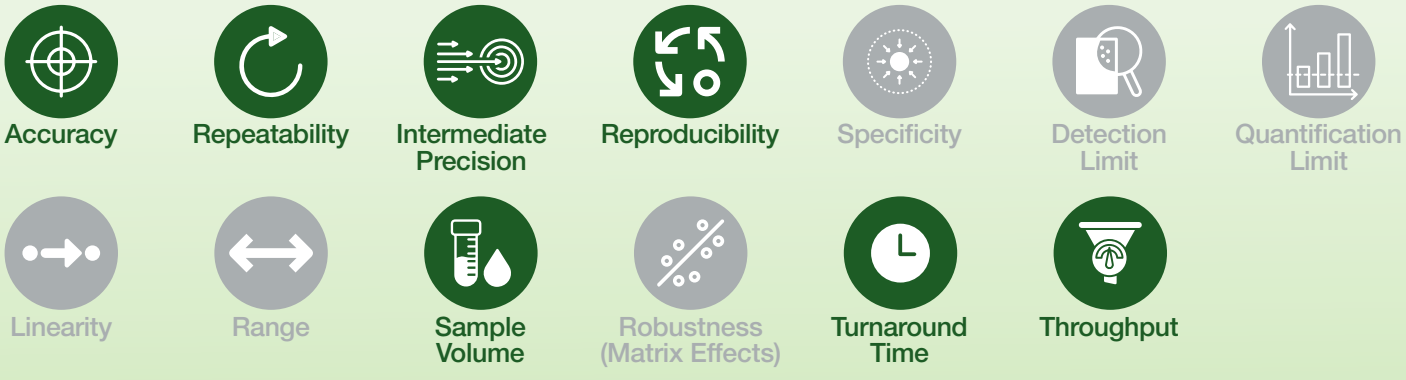
Analytical Methods for the Development and Manufacturing of Viral Vectors[1,2]

Summary of most common analytical methods for evaluating Critical Quality Attributes (CQAs) in therapeutic viral vectors for *in vivo* and *ex vivo* gene therapy

Property	Definition	Critical Quality Attribute (CQA)	Measured Element	Common Assessment Technique/Assay
Identity	Confirmation of the identity of the viral vector and its transgene	Vector identity	Transgene sequence in transduced cells	qPCR, sequencing (e.g., NGS)
Yield	Quantification of the viral particles	Physical viral titer	Viral proteins (e.g., p24), Viral RNA	ELISA, qPCR
		Vector Copy Number (VCN)	Genes specific to each vector type	qPCR
Potency	Assessment of the biological activity	Functional titer	Transgene copy number, transgene expression	Flow cytometry, qPCR
		Infectivity	Percentage of the viral vector that is able to transduce and deliver the transgene	Ratio between infectious and physical titer
Purity	Evaluation of the absence of product-related (e.g., partial or full empty capsids) or process-related (e.g., endonuclease, host cell proteins, host cell DNA) impurities.	Residual Host Cell Proteins (HCP)	HCP	ELISA
		Residual benzonase	Endonuclease (used to remove residual plasmid DNA)	ELISA
		Residual plasmid DNA	Genes specific to each vector type (e.g., VSV-G gene for lentivirus)	qPCR
Safety	Control of the presence of potentially immunogenic or toxic elements	Endotoxin	Endotoxin	Commercial kits with various readouts[3]
		Mycoplasma	Genes specific to Mycoplasma	qPCR
		Replication Competent Virus (RCV)	Genes specific to each vector type	qPCR and ELISA. Involves cell culture.

Critical Parameters in the Qualification and Validation of Analytical Methods for Therapeutic Viral Vectors[4]

Critical parameters that can be improved using automation are highlighted in dark green



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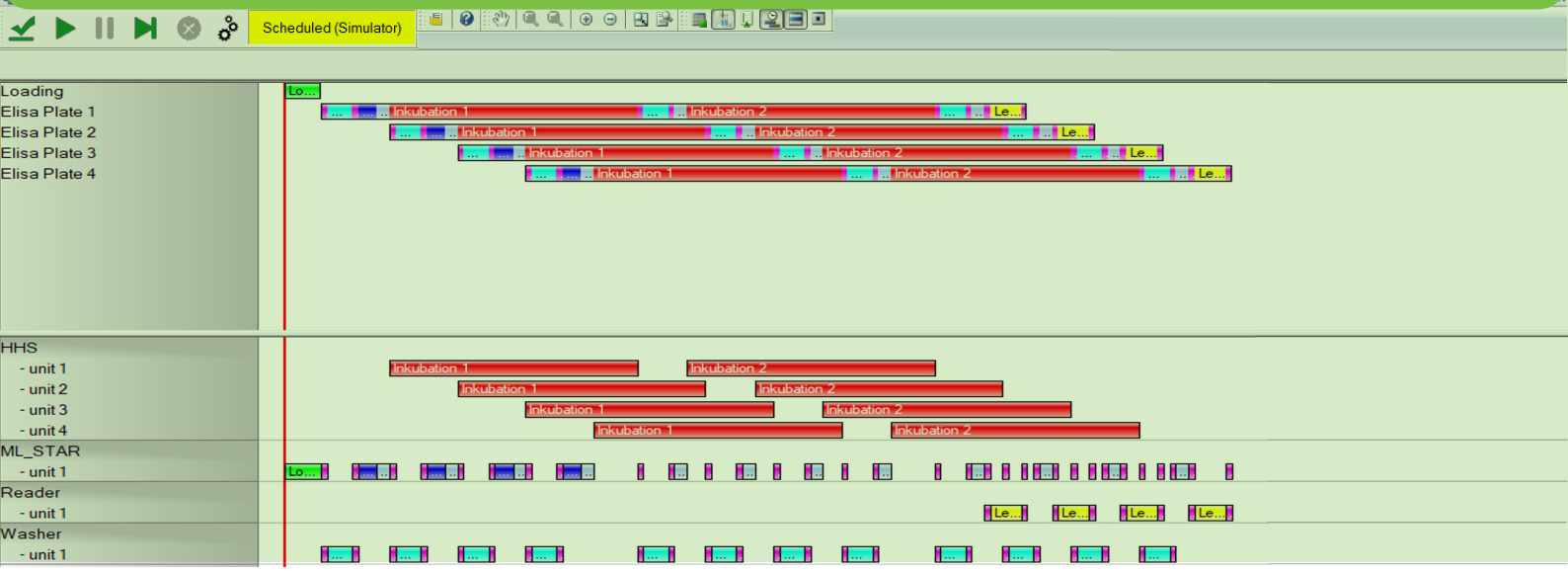


What Hamilton Can Offer

Hamilton Platforms

Hamilton Robotics specializes in developing, manufacturing and customizing automated liquid handling workstations.

To cover a broad spectrum of throughputs and integration capabilities, Hamilton Robotics offers three platform categories: (i) the Microlab® VANTAGE line for high-throughput assays (35-60 SBS plates) and 360° integrations, including Hamilton's Logistic and Rear Integration Cabinets, (ii) the Microlab® STAR™ and STAR V lines, for medium-to-high-throughput assays (25-60 SBS plates) and 180-270° integrations, and (iii) the Microlab® NIMBUS and Microlab® Prep™, for lower throughputs and entry-level users (7-20 SBS plates) (**Figure 1**). By Summer 2023, all Hamilton platforms in the medium- to high-throughput range will be equipped with the same Hamilton VENUS® software: Know ONE – Know ALL.

The platforms on our STAR™, STAR V and VANTAGE lines can be equipped with 96 or 384 Multi-Probe Heads (MPH), in addition to 2-16 single-channel pipettes. The ML NIMBUS line can also be equipped with a single 96 MPH.

In addition to liquid transfers, Hamilton platforms can perform a variety of steps (e.g., heating, shaking, cooling, capping, decapping, pumping media to the deck, centrifuging, pH measurement, filtration, solid phase extraction, image analysis, barcode and 2D code reading) thanks to the availability of multiple on-deck modules. Our projects in the pharmaceutical industry often include third-party integrations with plate readers, washers, incubators and imaging systems, among others.

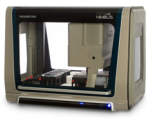
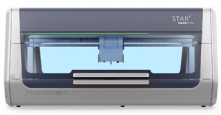
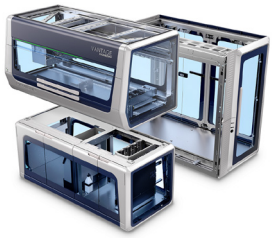
	Entry-Level Automation	High-Performance Benchtop Automation	High-Performance Automation
	 Microlab® Prep™  Microlab® NIMBUS™ line	 Microlab® STAR line  Microlab® STAR V line	 Microlab® VANTAGE line
Throughput and walk-away time	+	++	+++
Low volume pipetting	++	++ (+++ for ML STAR V)	+++
Method complexity	+	++	+++
Third-party integration	+	++ (180-270°)	+++ (360°)

Figure 1: Hamilton Robotics' Platform Lines.

Hamilton Systems and GMP Compliance

Many of the GMP requirements for automated methods are related to the software, particularly in reference to the FDA's Title 21 of the Code of Federal Regulations, Part 11, specific to electronic records and electronic signatures (21 CFR Part 11). These regulations aim to ensure that Electronic Records and Electronic Signatures (ERES) are trustworthy, reliable and equivalent to paper records.

All Hamilton platforms from the STAR line use a VENUS® software equipped with the necessary features for GMP compliance. By Autumn 2023, the STAR V and VANTAGE lines will also be offered with the same GMP-compliant software package, which includes:

1. **Controlled system access and training.** Each user is given a role to interact with the software depending on their qualification and training. The role defines the permissions and restrictions of the user (e.g., people with a specific role can only run experiments but not edit methods). Hamilton provides ISO-9001-compliant training with training certification for users (**Figure 2**).

2. **Sample traceability.** The movement of the sample throughout the entire workflow is monitored and reported.

3. **Time-stamped audit trails.** The VENUS® software provides a thorough report showing user identity, date, time and sequence of events. These records can be easily printed and are human-readable (**Figure 3**).

4. **Data integrity.** In addition to restricting unauthorized access (point 1), the VENUS® software detects external records alterations.

5. **System documentation.** Hamilton provides documentation following ISO 9001, including software manuals and technical and service bulletins. Additionally, Hamilton also provides key documents such as (i) Installation Qualification (IQ, showing that Hamilton equipment, as installed, complies with the approved design and the manufacturer's recommendations), and (ii) Operational Qualification (OQ, indicating that the Hamilton system operates according to its operational specification in the selected environment).

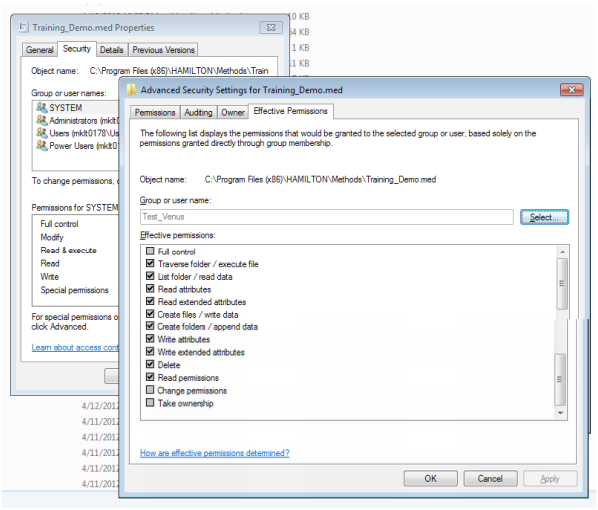


Figure 2: Set-up of Permissions and Restrictions for a User Group.

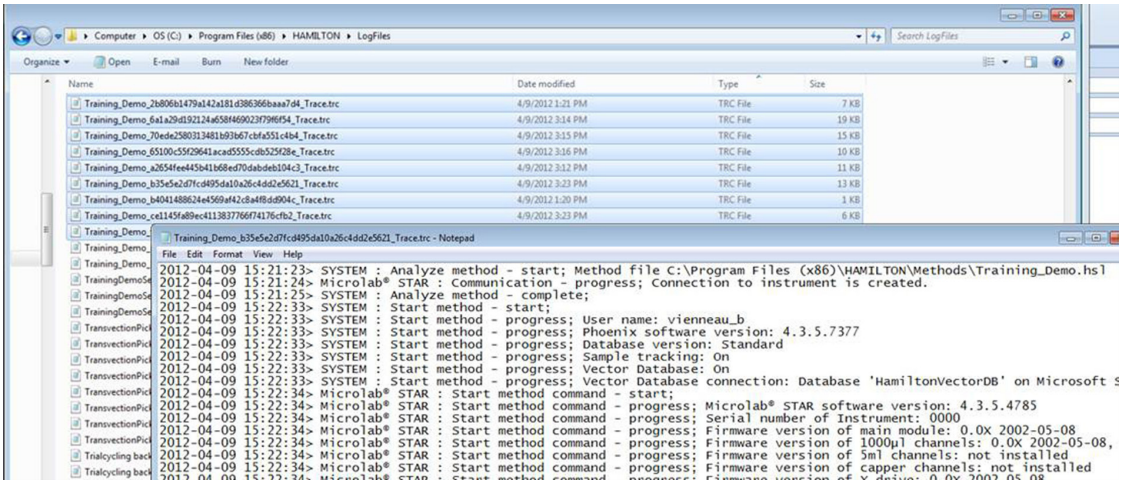


Figure 3: Trace File (.trc) Documenting Run Time Events for a Particular Method. *A unique trace file is generated for each run time event with date/time and user id information located at the end of the file.*

Hamilton Automated Solutions

Hamilton offers a wide range of solutions for various applications. From ready-to-use solutions (for fast implementation) to fully customized solutions explicitly designed to fulfill the needs of each specific project, Hamilton's philosophy is to provide customers with a broad range of options. In the following sections, we describe some of the ready-to-use and customized solutions that Hamilton offers for molecular, ligand-binding and cell-based assays.

Hamilton Automated Solutions for Molecular Workflows

Hamilton Robotics has developed various (Assay Ready) Workstations for Nucleic Acid (NA) extraction, qPCR/RT-PCR set-up and library prep for Next-Generation Sequencing (NGS). Our (Assay Ready) Workstations have standardized hardware and software packages, allowing us to quickly develop and qualify specific workflows. We offer four Assay Ready Workstations for NA extraction (the NIMBUS® Presto, the Long String STAR V, the MagEX STARlet and the Genomic STARlet 2.0), one workstation for (q)PCR (the PCR Prep STARlet), and four Assay Ready Workstations for NGS library prep (the NGS STAR, the Clinical NGS STAR, the NGS STARlet and the NGS STAR V). See **Table 1** for a detailed description of each of the eight systems. The figures of the individual deck layouts were previously described in our eBook Molecular Diagnosis of Infectious Diseases.

Hamilton Automated Solutions for Ligand-Binding Assays (LBAs)

Virtually all the sample-handling steps of most LBAs can be automated, including serial dilutions, heating, cooling, and shaking. An example of a configuration for the automation of an ELISA workflow can be seen in **Figure 4**.

Table 1: Hamilton (Assay Ready) Workstations for Molecular Workflows.

	Workflow Automated	Hamilton Platform	Technology	Throughput	Kit Providers from which there are available or qualifies methods	Instrument Classification
NIMBUS® Presto	NA extraction	Microlab NIMBUS® HD	- Magnetic disc-based - Magnetic bead-based (Thermo Fisher Scientific)	96 samples	PacBio, Promega, MACHEREY-NAGEL, Thermo Fisher Scientific, Omega Bio-Tek	RUO
Long String STAR V	NA extraction	Microlab® STAR V	Magnetic disc-based	24 samples	Bionano	RUO
MagEx STARlet	NA extraction	Microlab® STARlet	Magnetic bead-based	96 samples	MACHEREY-NAGEL, Thermo Fisher Scientific, Omega Biotek, Promega, Zymo Research, Molg3n	RUO
Genomic STARlet 2.0	NA extraction	Microlab® STARlet	Silica-based filter plate (MACHEREY-NAGEL)	96 samples	MACHEREY-NAGEL	RUO
PCR Prep STARlet	PCR set-up	Microlab® STARlet	-	96 samples	Thermo Fisher Scientific	RUO
NGS STAR	NGS library prep	Microlab® STAR™	Magnetic bead-based + On-Deck Thermal Cycler	48-96 samples	Illumina, Qiagen, Roche KAPA, New England Biolabs, Oxford Nanopore Technologies	RUO
Clinical NGS STARlet	NGS library prep	Microlab® STARlet	Magnetic bead-based + On-Deck Thermal Cycler	24 samples	Illumina	IVD-R*
NGS STARlet	NGS library prep	Microlab® STARlet	Magnetic bead-based + On-Deck Thermal Cycler	24 samples	Illumina	RUO
NGS STAR V	NGS library prep	Microlab® STAR V	-	96 samples	Roche KAPA, New England Biolabs, Illumina	RUO

** The Clinical NGS STARlet is an IVDR-classified and certified instrument. However, the full IVDR solution can only be provided in conjunction with IVDR-classified and certified partner reagents and methods.*

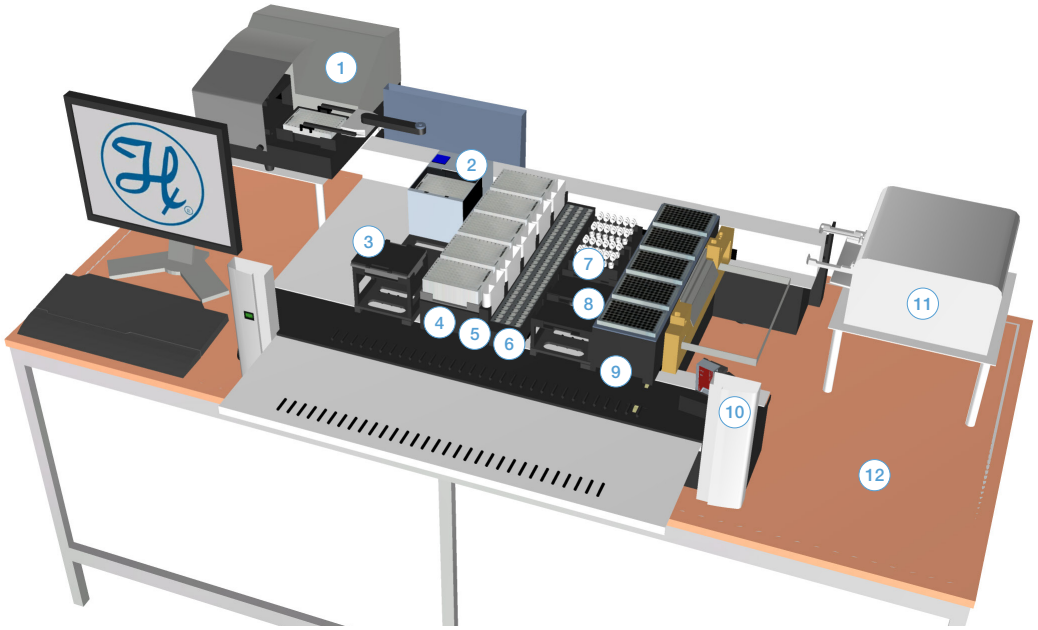


Figure 4: Example of a Configuration for the Automation of an ELISA Workflow. *(1) Plate Washer, (2) Cooling Module, (3) Lid Parking Position, (4) Deep Well Plate Carrier, (5) Trough Carrier, (6) Sample Carriers, (7) Microtube Module, (8) Microtiter Plate Modules, (9) Tip Carrier, (10) Barcode Reader, (11) Plate Reader, (12) Table.*

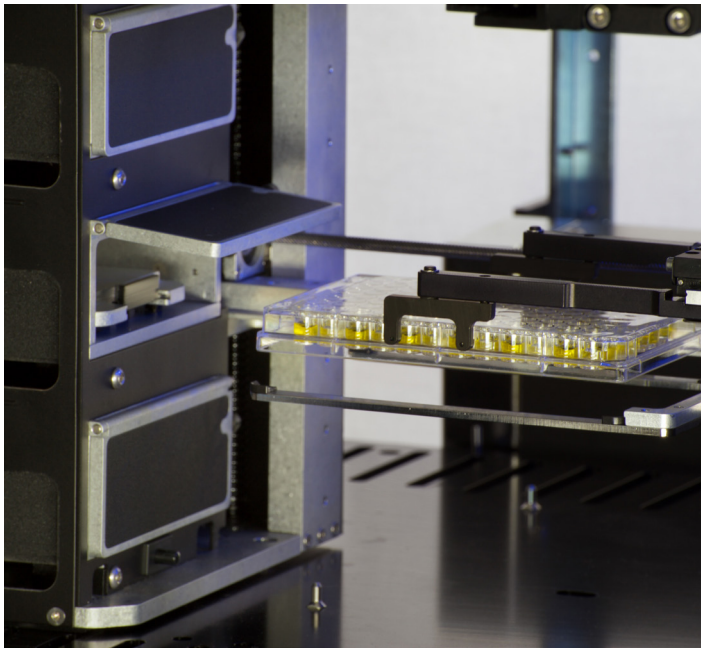


Figure 5: Hamilton Incubator Shaker Integrated Alongside Hamilton Automated Platforms.

For temperature-controlled enzymatic assays that require an even, temperate environment over the entire plate, Hamilton offers the Hamilton Incubator Shaker (HIS), which can be integrated alongside the platform deck (**Figure 5**). The HIS has four individual slots with separate doors that are accessed by a fork. The Incubator is compatible with SBS microplates, and each slot has independent temperature control with a range from ambient +4°C to +60°C. The optional shaking capabilities of the HIS make this module particularly attractive for ELISA workflows.

Hamilton also offers various deck modules with temperature control functionalities, including our Hamilton Heater Shaker™ (HHS), Heater Cooler™, Heating Cooling Module, and Hamilton On-Deck Thermal Cycler (ODTC). Additionally, Hamilton offers two dedicated Cooling Carriers to ensure that samples can be kept cool while on-deck (**Table 2**). These modules can also be used in temperature-steps of cell-based assays.

Integrations for Fully-Automated Workflows

Hamilton platforms can be integrated with third-party plate readers, plate sealers, plate peelers, decappers, washers and incubators, among others, to automate entire workflows (**Figure 6**). In addition, to facilitate running long, repetitive workflows, such as ELISAs, Hamilton VENUS® software includes schedulers. Scheduling allows for the optimization of resources and the running of several assays in parallel (**Figure 7**).

Hamilton Automated Solutions for Cell-Based Assays

As previously mentioned, some analytical methods used to assess viral vectors require preparatory assays that include (long-term) cell culture. Hamilton automated liquid handlers can perform some of the steps of these workflows (e.g., media exchange) or entire cell maintenance workflows by integrating third-party devices such as incubators and plate hotels. An example of a configuration for the automation of a workflow involving cell culture in microtiter plates can be seen in **Figure 8**.

Table 2: Hamilton Deck Modules and Carriers for Temperature-sensitive Steps.

	Hamilton Heater Shaker™ (HHS)*	Heater Cooler™	Heating Cooling Module	Hamilton On-Deck Thermal Cycler (ODTC)	Cooled Carrier	Sample Cooling Carrier
						
Temperature Range	Ambient + 5° C up to 105° C	0° C up to 110° C	4° C up to 95° C	4° C up to 99° C	-25° C up to 150° C	4° C up to ambient
Shaking Capabilities	Yes, up to 2500 rpm	No	No	No	No	No
External Chiller/ Cryostat	No	No	No	No	Yes	Yes

** Available on all four platforms*

Figure 6: Two Examples of the Integration of Hamilton platforms with Third-Party Instruments for LBAs. *Photos were taken by Hamilton Application Specialists during system installations.*

Figure 7: View from Hamilton's VENUS® Scheduler Showing the Planning of Four Parallel ELISA Workflows. *Each step is shown with a color code described in the caption at the bottom of the image.*

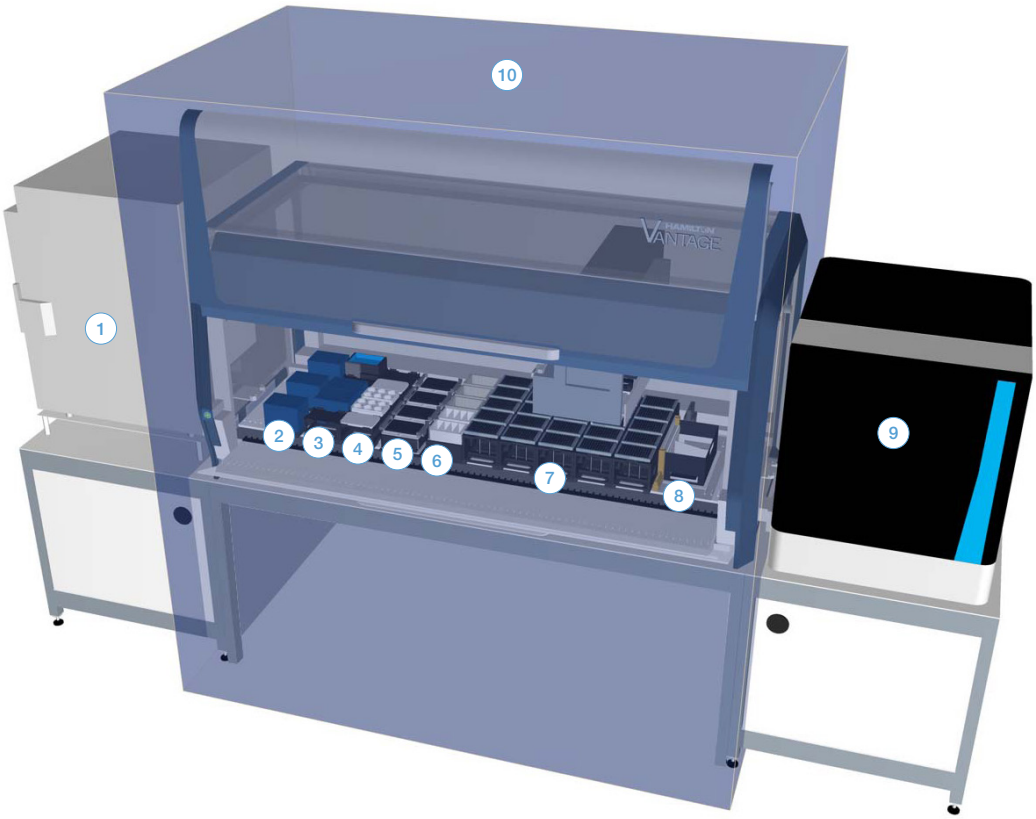


Figure 8: Example of a Configuration for the Automation of a Workflow Involving Cell Culture in Microtiter Plates.

(1) Plate Hotel, (2) Carrier with Media Line Modules with three troughs (shown in blue) and up to for six different medias with priming and washing, (3) Carrier with Liquid Dispenser (used as liquid waste module), 2X Plate Carriers and 2X Tilt Modules (shown in blue), (4) Plate Carrier, (5) Carrier with 4X Hamilton Heater Shakers™, (6) Cooled/heated Carrier for reagents, (7) 5X Tip Carriers, (8) Waste, (9) Plate Incubator, (10) Bio Safety Cabinet Class 2 enclosing the Hamilton Liquid Handler (positions 2-8).

Hamilton Robotics offers various modules to facilitate the handling of cell cultures. Notice that most of the modules described in this section are only available on the STAR™, STAR V and VANTAGE lines.

Cell Handling

Labware Format

Hamilton automated systems are designed to handle suspension and adherent cell cultures on a wide range of SBS format labware, including 1, 6, 12, 24, 48, 96-well plates, standard or customized.

Adherent cultures growing in other types of labware (e.g., T75 Flasks) are often detached and resuspended in a suitable concentration on a separate laminar flow cabinet or biosafety cabinet before being brought into the Hamilton platform for automated seeding. In these cases, the cell solution is often transported in plastic tubes with screwtop caps (e.g., Falcon Tubes). Hamilton Twister Channels and Decapper Modules were specifically developed to handle these tubes (i.e., diameters between 15 mm and 38 mm), including their tr,ansport, vortex (up to 1500 rpm +/-5%), decapping and recapping. The Twister Channels are also equipped with 1D Barcode Readers (**Figure 9**).

Pipetting

Hamilton platforms can be equipped with channels that allow for the pipetting of a variety of volumes; from 350 nl to 5 ml. The gentle handling of cells is ensured through Hamilton's proprietary pipetting technologies: Compressed O-Ring Expansion (CO-RE®), for tip attachment and positioning; Liquid Level Detection (LLD), for detection of the exact level of liquids in tubes or plates; and Monitored Air Displacement (MAD) and Total Aspiration and Dispensing Monitoring (TADM™), for the dynamic tracking of each aspiration and dispensation step. Furthermore, Hamilton channels allow for asymmetrical spreading and independent volume handling and movement on the Z plane, which is particularly beneficial for handling different plates in parallel. Some Hamilton platforms can also be equipped with MagPip Channels. MagPip is a new pipetting technology, based on the movement of a magnetized piston inside a tube's magnetic field, and it can cover a wide volume range (350 nl-750 µl) at the highest level of precision.

The parameters for the aspiration and dispensing of liquids using Hamilton channels can be adjusted on Hamilton VENUS® software to ensure the desired flow rate and tip positioning (e.g., distance from the bottom of the well and side touch for dispensing on the well's wall, among other parameters) (**Figure 10**). On the Microlab® Prep™ software, the liquid classes are already set up and can be further customized.

A common challenge during the automation of the aspiration steps on cell culture methods is the complete removal of the media without touching the bottom of the well. To accomplish this task, Hamilton offers the Tilt Module, which mimics the manual tilting of the microplate, facilitating the complete removal of the media from the wells in 1-24-well plates (**Figure 11**).

Figure 9: Twister Channel and Decapper Module Integrated into Hamilton Automated Platforms.

A

B

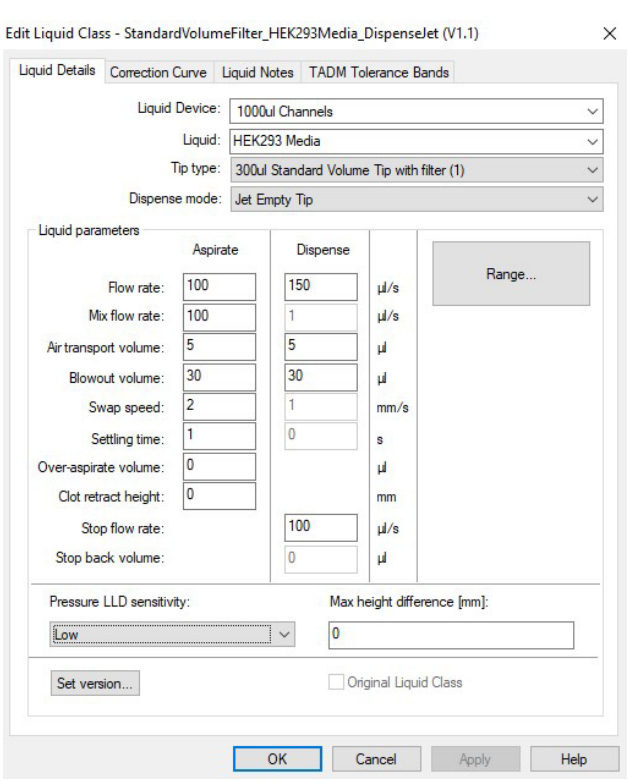
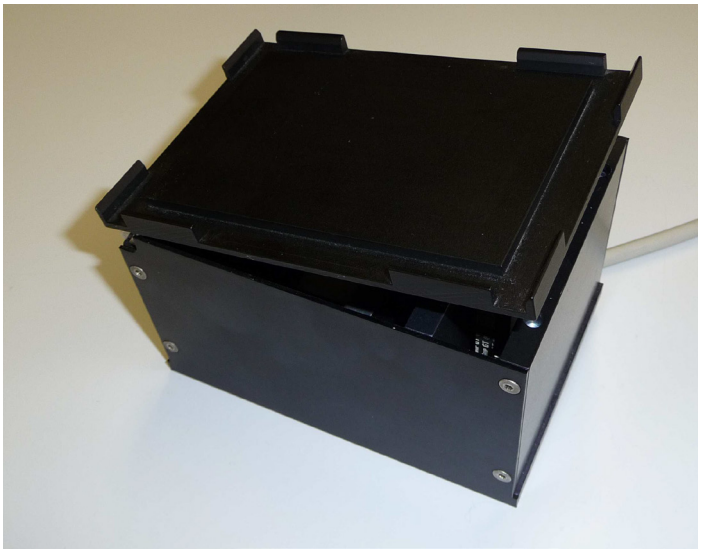


Figure 10: View from Hamilton's VENUS® Software. *The images indicate some of the aspiration and dispensing parameters that can be adjusted during method development.* ***A.*** *Screenshot from Power Step method development.* ***B.*** *Screenshot from the Liquid Class Editor*



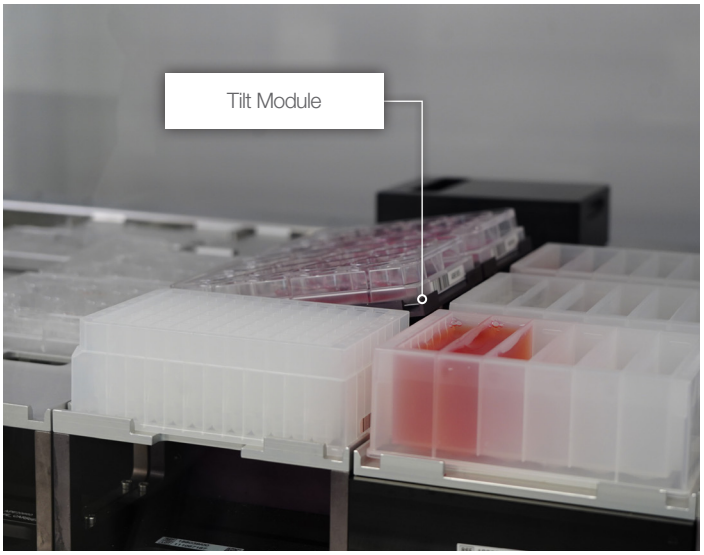


Figure 11: Tilt Module and Tilt Module in Action. *The photo on the right was taken by Hamilton Application Specialists during system installations.*

Media Supply and Removal

In order to facilitate the bulk supply of media onto the deck, Hamilton platforms can be equipped with Media Line Modules. Each Media Line Module includes a Peristaltic Pump, a selection valve for up to three source liquids and a Deck Trough Module equipped with a Liquid Level Sensor, a Priming Station, an Overflow Drain, and a movable Tubing Guide that allows for automated replacement of the trough. The module has a source tubing connected to the media sources, and a waste tubing connected to a waste/overflow container (**Figure 12**). All tubing is replaceable. The media can be pre-warmed on a Pre-Heating Module.

Non-sterile bulk supply or removal of liquid onto and out of the deck can be done using Hamilton Multi-Flex (MFX) Liquid Dispenser 2.0. The MFX Liquid Dispenser 2.0 consists of a main module with a valve/syringe combination to pump liquid out or into up to two containers and a deck trough module (**Figure 13**).

System Sterility

The most basic technology to prevent contamination in Hamilton's liquid handlers is its pipetting technology. Hamilton's pipetting technology is based on air displacement rather than liquid displacement, avoiding the use of liquids where microorganisms naturally grow and the direct contact between the sample and the plunger.

In order to ensure the sterility of the system beyond the control of pipetting-induced risks, Hamilton platforms can be equipped with High-Efficiency Particulate Air (HEPA) Filter Hoods and UV Lamps (**Figure 14**).

Figure 12: Close-up view of two Media Line Deck Modules. *The photo was taken by Hamilton Application Specialists during system installations.*

Figure 13: Multi-Flex (MFX) Liquid Dispenser.

Figure 14: Four Examples of System Configurations Ensuring Sterility of the Automated Workflow. *The two upper photos show Hamilton systems inside Class II BSCs, and the two lower photos show Hamilton systems with HEPA Hoods. Photos were taken by Hamilton Application Specialists during system installations.*

Hamilton also offers a variety of consumables suitable for tissue culture, including several types of sterile tips, either with or without filter. Our Sterile Filtered Tips Boxes are lidded and individually wrapped to maintain sterility and protect tip integrity (**Figure 15**). Tip boxes can be stacked in the Microlab® VANTAGE Entry/Exit modules or in Plate Hotels and Stackers (including Hamilton's Verso Q10 Plate Hotel), freeing up precious deck space.

In addition to tips, Hamilton offers tubes, plates and reagent containers. Hamilton consumables are produced in an ISO Class 8 Cleanroom, based on ISO 14644 standards as well as ISO 9001 and ISO 13485. Most consumables meet the European Commission (EC) directives and Conformité Européenne (CE) and *In Vitro* Diagnostics (IVD) regulations.

Integrations for Long-Term Cultures

As previously described, Hamilton platforms can be integrated with third-party devices (e.g., incubators, plate hotels, and cell imaging systems) for full walk-away, automated, cell-based assays (**Figure 16**).

Moreover, Hamilton offers CECULA, a dedicated software for cell culture planning. CECULA'S Graphical User Interface (GUI) is very easy to use and satisfies the requirements of workflows with long-term cultures over multiple weeks.

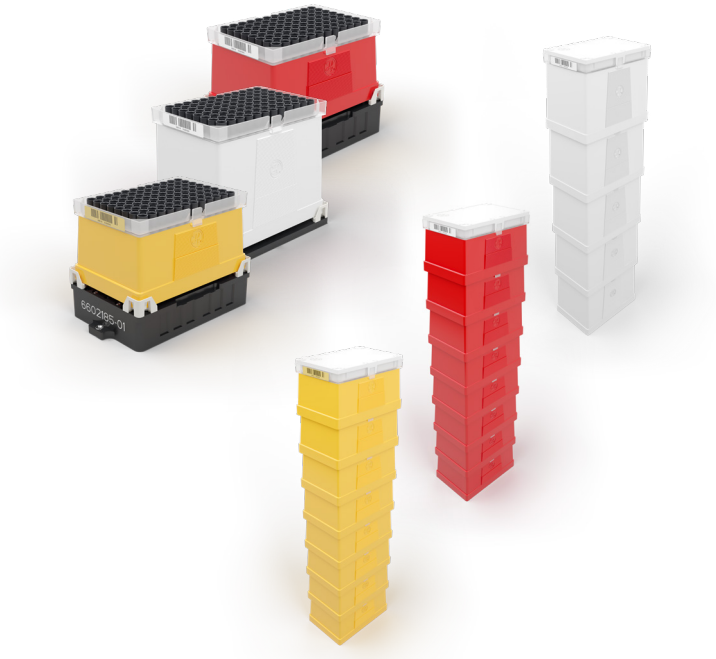


Figure 15: Hamilton Sterile Filtered Tips Boxes. *50 µl (red), 300 µl (yellow) and 1000 µl (white) Sterile Filtered Tips Boxes are shown individually on top of deck adaptors on the left-hand side and in stacks on the right-hand side.*

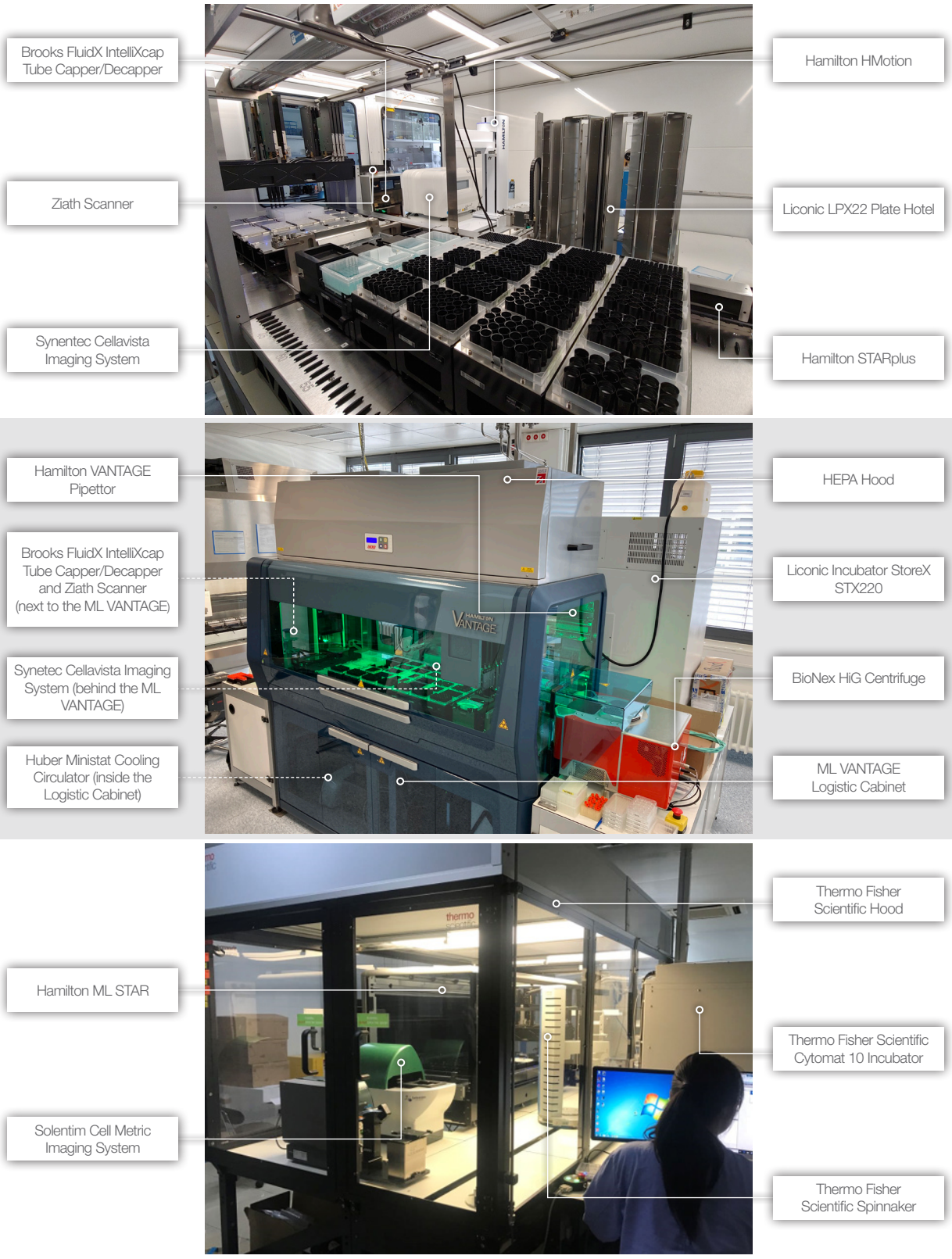


Figure 16: Three Examples of Integrations of Hamilton platforms with Third-party Instruments for Cell-Related Assays. *Photos were taken by Hamilton Application Specialists during system installations.*

CECULA expands the capabilities of Hamilton's standard VENUS® software to allow for changes in the workflows during ongoing experiments and the running of several batches in parallel. Similar to the VENUS® scheduler, CECULA calculates the system's resource consumption to warn the user about upcoming shortages (e.g., an insufficient number of pipetting tips before the weekend). The software also estimates each workflow's time, helping in the planning of further experiments and allowing for optimal system utilization (**Figure 17**).

Other Solutions

Hamilton also offers dedicated solutions for sample aliquoting and sample storage. These solutions are described in our eBook Sample Processing and Storage for Biobanking.

Contact your local Hamilton representative to learn more about the configurations that are more suitable to your needs.

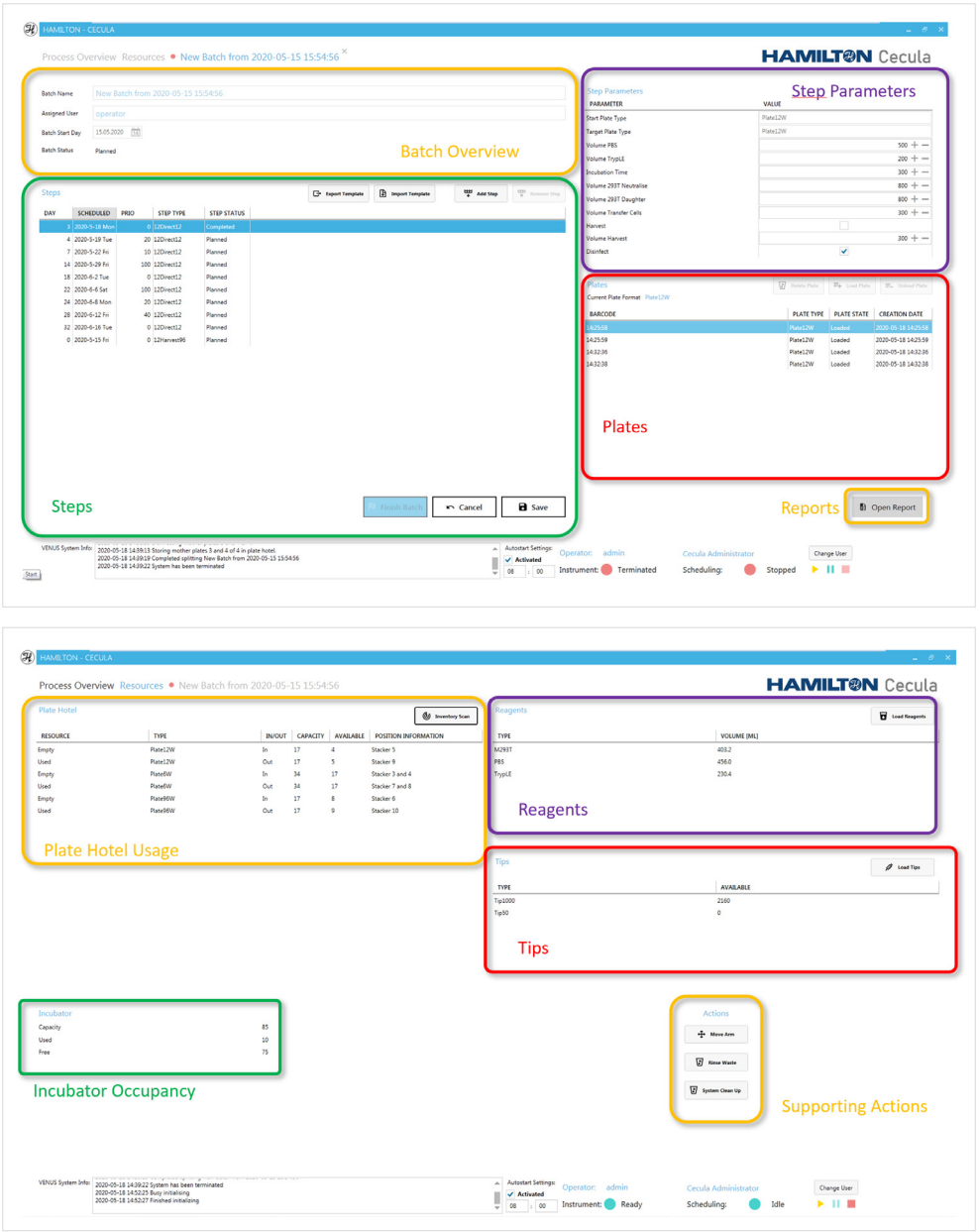


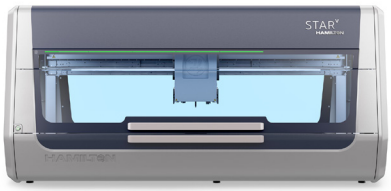
Figure 17: View from Hamilton CECULA V2.4 Software. *The images show a one-month batch management for passaging HEK293 cells (top image) and an overview of the resources (bottom image).*

HAMILTON

PLATFORMS
OUR PORTFOLIO
OF AUTOMATED LIQUID HANDLERS

Microlab® VANTAGE line

The next generation of liquid handling automation



Microlab® STAR^{V} Line

A new product class that combines the strength of the ML VANTAGE and the ML STAR™

Microlab® STAR™ line

The new version of the classic Hamilton automated liquid handler. As good as ever, only better.

Microlab® NIMBUS line

A compact, multi-channel automated liquid handler

Microlab® Prep™

Our smallest footprint liquid handler

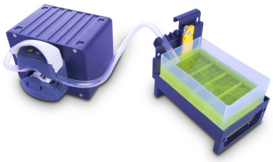
Please contact your local sales representative for more information.

HAMILTON

Tools for **Cell Biology** Applications

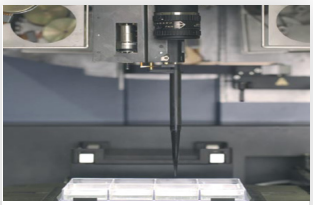
HAMILTON'S SOLUTIONS FOR EVERY NEED

CECULA Software
Dedicated software for cell culture planning



Media Line
For sterile bulk supply of media onto the deck

Liquid Dispenser
For bulk supply of media onto the deck and retrieval of liquid waste



EasyPick
For the identification, selection and picking of colony-forming structures

Hamilton Heater Shaker
For heating up to 105°C and shaking up to 2,500 rpm

Hamilton Heater Cooler
For cooling and heating between 0°C and 110°C

Cooling Carrier
SBS format carrier with a temperature range between -25°C and 115°C

Sample Cooling Carrier
Tube carrier with a temperature range between 4°C and ambient

Heating Cooling Module
For homogeneous cooling and heating between 4°C and 95°C

Clean Air Protection
Delivers filtered air to the inside of the liquid handler via a HEPA Filter

Twister Channel and Decapper
For transport, vortex, decapping and recapping of falcon tubes

bioREACTOR 48*
8-15 ml bioreactors with automated feeding, pH adjustment, induction and sampling on-demand

Tilt Module
For controlled tilt of plates to facilitate liquid removal

Tips Boxes with Filtered Tips
Lidded and individually wrapped to maintain sterility

Wide Bore Tips
With large orifices that facilitate the handling of 3D structures

*Integration from partners' technologies:

2mag magnetic motion

Please contact your local sales representative for more information.

HAMILTON

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