HAMILTON

Liquid Chromatography-Mass Spectrometry (LC-MS)

Analysis in Therapeutic Drug Monitoring

'The second eBook of the Hamilton series'

Updated - Version 2024

Foreword

The efficacy and clinical utility of Therapeutic Drug Monitoring (TDM) relies on the specific, sensitive, and reproducible measurement of analytes. Liquid Chromatography coupled with Mass Spectrometry (LC-MS) has emerged as an attractive alternative to traditional immunoassays. Currently, hundreds of therapeutic drugs are analyzed daily in the clinical setting, using this technique and several *in vitro* diagnostic (IVD)-certified kits have entered the market.

We believe that LC-MS has the potential to become the gold standard for TDM, and therefore we have collaborated with leaders in the field to ensure our automation solutions are compatible with their kits, workflows and instruments.

In this eBook, we discuss the impact of LC-MS in TDM, the drivers behind the need for automation during sample preparation for LC-MS, and the solutions that Hamilton offers to customers working in this field.

This eBook is part of a dedicated campaign for LC-MS analysis in TDM, where we aim to provide our readers with interesting educational resources and additional insights into the way our customers are using Hamilton solutions to accomplish their tasks.

I want to thank our Senior Market Segment Leader Analytics and Chromsystems for their valuable contributions to this eBook. We hope you find the content beneficial.

Your kind feedback is always highly appreciated.

Yours sincerely,

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Exploring the Role of LC-MS in Therapeutic Drug Monitoring

Therapeutic Drug Monitoring (TDM) refers to the repeated measurement of an analyte (a therapeutic drug) in a patient's blood or plasma, with the aim of finding the dosage regimen needed to maintain therapeutic concentrations. This methodology is fundamental in clinical pharmacology, an area that studies the effects of the body on the drugs (pharmacokinetics) and the effects of drugs on the body (pharmacodynamics).[1] TDM is generally required for drugs with a narrow therapeutic index and/or severe side effects such as immunosuppressants.[2]

For drugs with a narrow therapeutic index, small variations in dose or blood concentration may cause therapeutic failures or adverse drug reactions.

Historically, immunoassays have been the gold standard for the analysis of molecules in clinical chemistry. These tests are based on antibody–antigen binding; however they are subject to several limitations[3] such as: (1) limited specificity, due to cross-reactions; (2) limited reproducibility, due to antibody lot-to-lot variations from the same manufacturer and/or discordance between the results obtained from assays from different manufacturers; and (3) limited sensitivity, due to the low linear dynamic range. Furthermore, since every analyte requires a specific antibody, many do not have a commercially available immunoassay.

Liquid Chromatography coupled with Mass Spectrometry (LC-MS) has emerged as an attractive alternative to traditional immunoassays and other popular enzyme-subtract tests, as it does not suffer from the same limitations found in immunoassays. LC-MS is highly (1) specific and (2) reproducible, as it identifies compounds based on their unique chemical structure without the need to use antibodies, as well as (3) sensitive, due to its high linear dynamic range. These unique features enable the detection of steroids and other analytes that are difficult to measure at low concentrations. Additionally, LC-MS allows for the simultaneous analysis of several (up to hundreds) compounds from the same sample during the same run.[4,5,6] All these advantages have made LC-MS very attractive in the clinical setting, where thousands of samples (blood, plasma, serum, urine, hair, spittle and milk) are analyzed every year for the assessment of multiple parameters.[7]

The initial adoption of Mass Spectrometry (MS) in the clinical setting was limited by the technical complexities of the MS technique used between the 1980s and 1990s (mainly Gas Chromatography–Mass Spectrometry or GC-MS). The new (soft) ionization techniques, the high performance of the system once coupled with front-end Liquid Chromatography (LC) and a second mass analyzer (MS/MS), gave MS the conditions necessary to be a viable and attractive alternative for routine clinical laboratories.[8]

Initially, the use of LC-MS in TDM was focused on immunosuppressive drugs (also known as immuno-suppressants), however its use has now extended to the assessment of anticonvulsants, antibiotics, antifungal drugs, antiviral drugs, antidepressants, anti-cancer drugs and cardiac drugs, among others.[6,11] LC-MS is particularly well-suited for the clinical setting when using tandem mass spectrometry (LC-MS/MS) and performing Selected/

Multiple Reaction Monitoring (SRM/MRM), a technique that selects the analytes to be analyzed and quantified with high precision.[9] LC-MS/MS (particularly triple quadrupole MS) is currently one of the most popular methods for TDM.

Laboratories select clinical tests based on their individual requirements. From a regulatory point of view, it is not uncommon for clinical laboratories to use Laboratory-Developed Tests (LDTs, also known as "in-house tests" or "home brew tests"), which — unlike *in vitro* diagnostics (IVD)-certified tests — do not need US Food and Drug Administration (FDA) clearance or a European CE-IVD mark.

An LDT is an *in vitro* diagnostic test designed, manufactured and used within a single laboratory.[12] Their use in the clinic is expected to be limited in the future. By contrast, an IVD-certified test is an ***in vitro*** diagnostic test "sold as a complete kit that a laboratory purchases from a manufacturer, and comes with all of the procedures and controls to perform the test".[13] There are several commercial CE-IVD-certified tests for TDM on the market. However, most automated methods are currently run as LDTs.

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LC-MS FOR THERAPEUTIC DRUG MONITORING IN THE CLINICAL SETTING

Therapeutic Drug Monitoring (TDM) is a clinical practice used to optimize individual dosing regimens by measuring specific drugs at designated intervals to maintain their concentration within a target range.[1,2] The need for accurate, precise, and standardized measurement of drugs presents a major challenge for clinical laboratories and the diagnostics industry.[3]

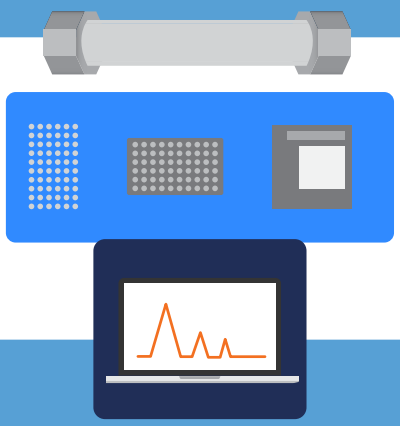
To be suitable for TDM, a drug should satisfy certain criteria, including:

- A narrow therapeutic target range
- Significant pharmacokinetic variability
- A reasonable relationship between blood concentrations and clinical effects
- Established therapeutic range and toxic threshold
- Availability of cost-effective drug assays

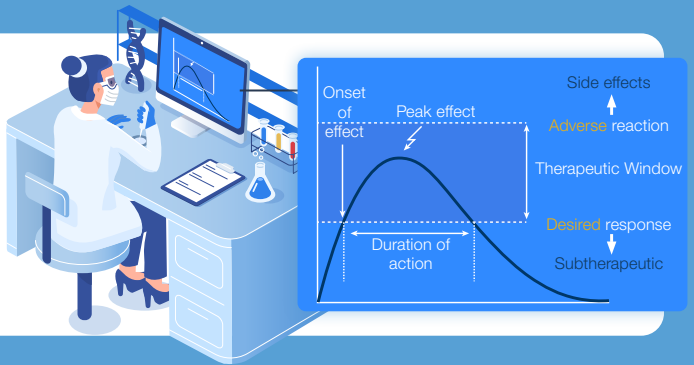
1 A diagnosis is made and a drug is selected. A dosage schedule is designed to reach a target therapeutic plasma concentration. The drug is administered to the patient.

2 Samples are collected from the patient at repeated intervals and tested in a clinical laboratory using Liquid Chromatography coupled with Mass Spectrometry (LC-MS).

**Please note that other analytical methods also exist.*



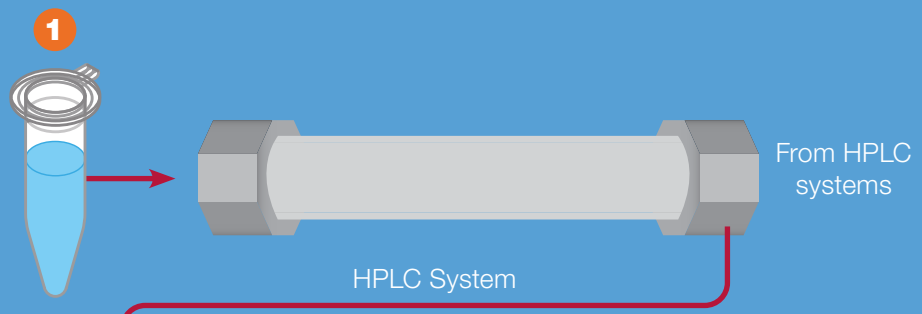
3 Drug concentration in the patient's blood/ plasma is determined. A pharmacokinetic model is applied and clinical judgement is used to adjust the dosage if necessary.



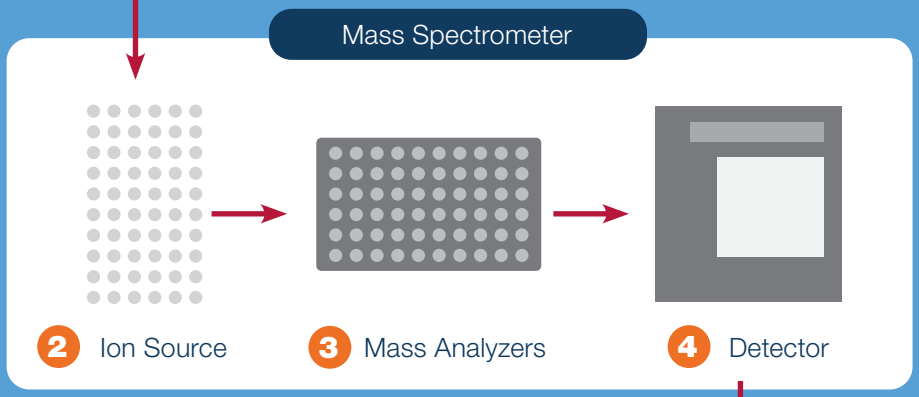
WHAT IS LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY?

Liquid Chromatography coupled with Mass Spectrometry (LC-MS) is a highly specific, sensitive and reproducible analytical technique that combines the physical separation capabilities of Liquid Chromatography (LC) with the mass analysis capabilities of Mass Spectrometry (MS).[4]

1 Samples are extracted/ prepared for analysis and then separated using a High-Performance Liquid Chromatography (HPLC) column.[5]



2 The separated sample species are sprayed into an ion source while transitioning into a gas phase.[5] Samples can be ionized through several methods. The most common for TDM analysis is Electrospray Ionization (ESI)



3 Mass spectrometry separates gas phase ions according to their m/z (mass to charge ratio) value, using electrical and/or magnetic fields to differentiate ions.[5,6] There are different types of mass analyzers. One of the most common for TDM analysis is the triple quadrupole.

4 As ions cross the mass analyzer, the detector counts them and amplifies their signal.[5]

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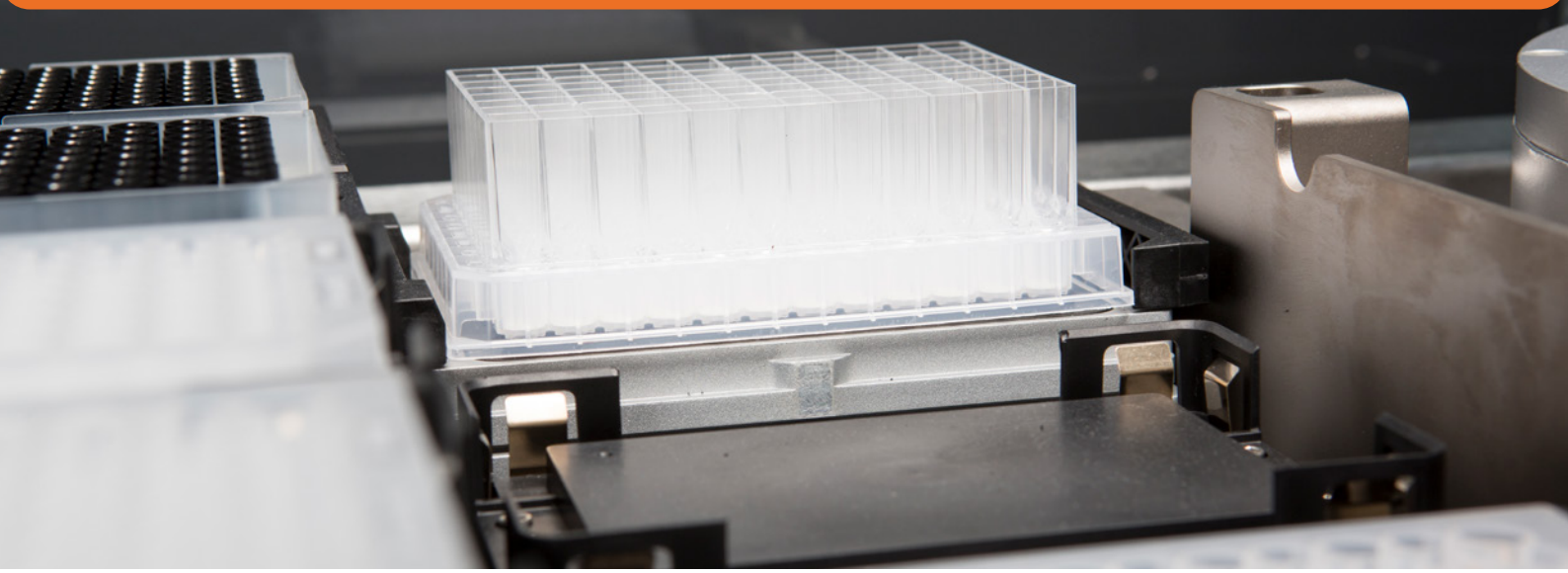

LC-MS: Why Do We Need Automation?

There has been a rapid adoption of LC-MS-based testing in the clinical setting over the past few years. Central laboratories, such as the University Hospital of South Manchester, UK, process between 2,000 and 3,000 samples per day (analyzing several analytes per sample).[1,2] Automation, particularly during the sample preparation step, can significantly increase the throughput of clinical laboratories and provide researchers with more time to focus on more valuable tasks.

Sample preparation is a critical step in LC-MS methodology. Due to the specific technology used for detection, mass spectrometers are very sensitive to the presence of salts and charged ions. Additionally, the detection of low-abundance molecules can be masked by the presence of high abundance ones.[3] The quality and reproducibility of the sample preparation step therefore has a significant influence on the final results. This is particularly critical in the clinical setting, where the results obtained from the tests have a direct influence on the patient's treatment.

There is no standard or universal method for the preparation of samples for LC-MS analysis in the clinical setting. Depending on the type and concentration of the analyte, the matrix, the goal of the assessment and the analytical method used, samples might require a very different treatment (e.g. reduction and alkylation, digestion, desalting, protein-precipitation, solid-phase extraction, etc.).[4,5] In the routine clinical setting, the workflows for each type of measurement need to be standardized to ensure reproducibility. Yet this is often hindered by manual processing errors. Even with the availability of experienced, qualified staff (which is an important source of fixed costs), inter-operator variability remains. While high-throughput is considered the main selling point of automation, in the clinical practice, the need for high reproducibility is the main reason for transitioning from manual to automated workflows.

Current regulatory changes also require a higher level of compliance for the diagnostic tests performed in the clinical setting (beyond investigational use), including LC-MS-based tests. At present, most clinical laboratories develop and validate their LC-MS assays as LDTs. In the US, laboratories with a Clinical Laboratory Improvement Amendments (CLIA) license are allowed to develop and use their own assays, without the need to obtain an IVD certification.[6] Even though the FDA has expressed the intention to better regulate LDTs, no final guidance has been issued since the initial draft guidance of 2014.[7] It must be highlighted, however, that despite the lack of final guidance, the FDA can, and already does, exercise "enforcement discretion" when there are concerns about the validity of the results provided by an LDT.[6]

In the European Union (EU), and since May 2022, a new regulation (EU 2017/746) came into force which changed the regulatory classification of *In Vitro* Diagnostic (IVD) tests and introduced stricter compliance measures (IVDR).[9] These measures cover the tests, kits, instruments, and users. Automation will help clinical laboratories comply with these new regulations to ensure:

- Traceability
- Standardization of workflows
- Elimination/minimization of human error

- Control of user access
- Workflows for handling errors

Reimbursement policies are expected to be based on the use of IVD tests complying with the new regulations. Many laboratories need to seriously evaluate existing instrument providers on regulatory grounds, as this will impact the complexity of any future LDT validation process.

"Platform selection is one of the most critical choices a diagnostic developer can make because it is effectively a partner for life".

– Hannah Mamuszka, CEO, Alva 10[6]

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ENSURING COMPLIANCE IN TDM THROUGH AUTOMATION

Liquid Chromatography-Mass Spectrometry (LC-MS) based testing is increasing in popularity for clinical laboratories conducting Therapeutic Drug Monitoring (TDM). In response to an increase in this and other types of clinical tests, European regulations for diagnostic tests performed in the clinic have recently changed (from IVDD to IVDR). Stricter regulation measures are also still in discussion in the US.

Clinical laboratories need to adapt to stricter regulatory control for Laboratory Developed Tests (LDTs), which cover the tests, kits, instruments, and users.

Currently, most clinical laboratories develop and validate their own LC-MS assays.

In the US, laboratories with a Clinical Laboratory Improvement Amendments (CLIA) license can develop and use their assays, without the need to obtain an IVD certification.

In 2017, Europe introduced the EU 2017/746, which came with new, harsher regulatory measures. These new regulations are applicable from 2022 onwards.

In Europe, LDTs can be developed and used in certified labs, when no comparable commercial kit is available. LDTs cannot be distributed commercially.

Automation can be introduced to clinical laboratories to help optimize their testing and increase compliance with these regulations, providing:

- Better (end-to-end) traceability
- Standardization of workflows
- Elimination/minimization of human error
- Controlled user access
- Robust workflows for handling errors
- Increased throughput
- Better reproducibility

What Can Hamilton Offer?

Hamilton is a market leader in precision liquid handling, where innovation is not only a key internal value, but also a value that we strongly support in the fields we serve. Sample preparation for LC-MS is one our focus areas. LC-MS has the potential to become the gold standard for TDM, and we have collaborated with many leaders in this field to ensure our solutions are compatible with their kits, workflows and instruments.

Our solutions are designed with laboratory needs in mind and our philosophy is to provide our customers with the widest range of options — from turnkey to customized solutions, and from IVDR-certified solutions to solutions supporting the automation of LDTs. Our most popular sample preparation workflows are designed on our Microlab® STAR™ line; however, our solutions can be adapted to any of our other platforms.

For customers seeking IVD solutions

Together with Chromsystems and Shimadzu, Hamilton has developed two CE-IVD-certified automated solutions for clinical LC-MS sample preparation: The MassSTAR (**Figure 1**), and the STAR CL (**Figure 2**). The MassSTAR is based on a Hamilton Microlab® STARlet, and it is validated to perform automated sample preparation with Chromsystems' TDM kits: ***Mass****Tox*® Immunosuppressants (to measure Cyclosporin A, Everolimus, Sirolimus and Tacrolimus) and ***Mass****Tox*® TDM Series A kits (to measure more than 100 parameters without column switching or change of the mobile phases). The STAR CL is based on a Hamilton Microlab® STAR, and it is validated to perform automated sample preparation with Alsachim's (Shimadzu) DOSIMMUNE™ kit (to measure Cyclosporin A, Sirolimus, Everolimus and Tacrolimus).

We are currently working with other partners to automated more workflows.

For customers looking for RUO solutions

For customers interested in automating their own solutions starting from a pre-defined layout, we offer the Dual MassSTAR (**Figure 3**), which is an open platform. Our collaborators at Labor Berlin have validated workflows for the analysis of several molecules relevant for TDM (antibiotics, antidepressants, antiepileptics and neuropileptics) using this platform. We can replicate or adapt these conditions and layouts for our customers to allow for a more rapid implementation.

We know that each customer is different and we aim to provide solutions for individual needs. We can provide fully customizable deck layouts, including direct sample injection into the LC-MS and integration of our various standard modules for incubation, shaking, centrifugation and Solid-Phase Extraction (SPE), as well as integration with third-party devices. One of our most popular modules is the Monitored Multi-flow, Positive Pressure Evaporative Extraction – [MPE]$^{2®}$ – for SPE (**Figure 4**). The positive pressure system of our [MPE]$^{2®}$ maintains equal pressure across the filter plate, eliminating the path of least resistance. Furthermore, the [MPE]$^{2®}$ can be ordered with the complementary reagent dispenser to automatically add liquid to the plate, as well as with the complementary evaporator to dry down samples with a heated gas. We also have a vacuum-based SPE module for customers preferring this solution.

For customers working with lower throughputs, we offer our FlipTube solutions (**Figure 5**). Instead of using plates, samples can be processed in 1.5 ml FlipTubes specially designed with a unique lid that prevents contamination and evaporation. Our platforms can be equipped with the FlipTube™ tool-set that automates the opening and closing of the FlipTubes, as well as with transport tools, racks, adaptors, carriers and dedicated shakers and

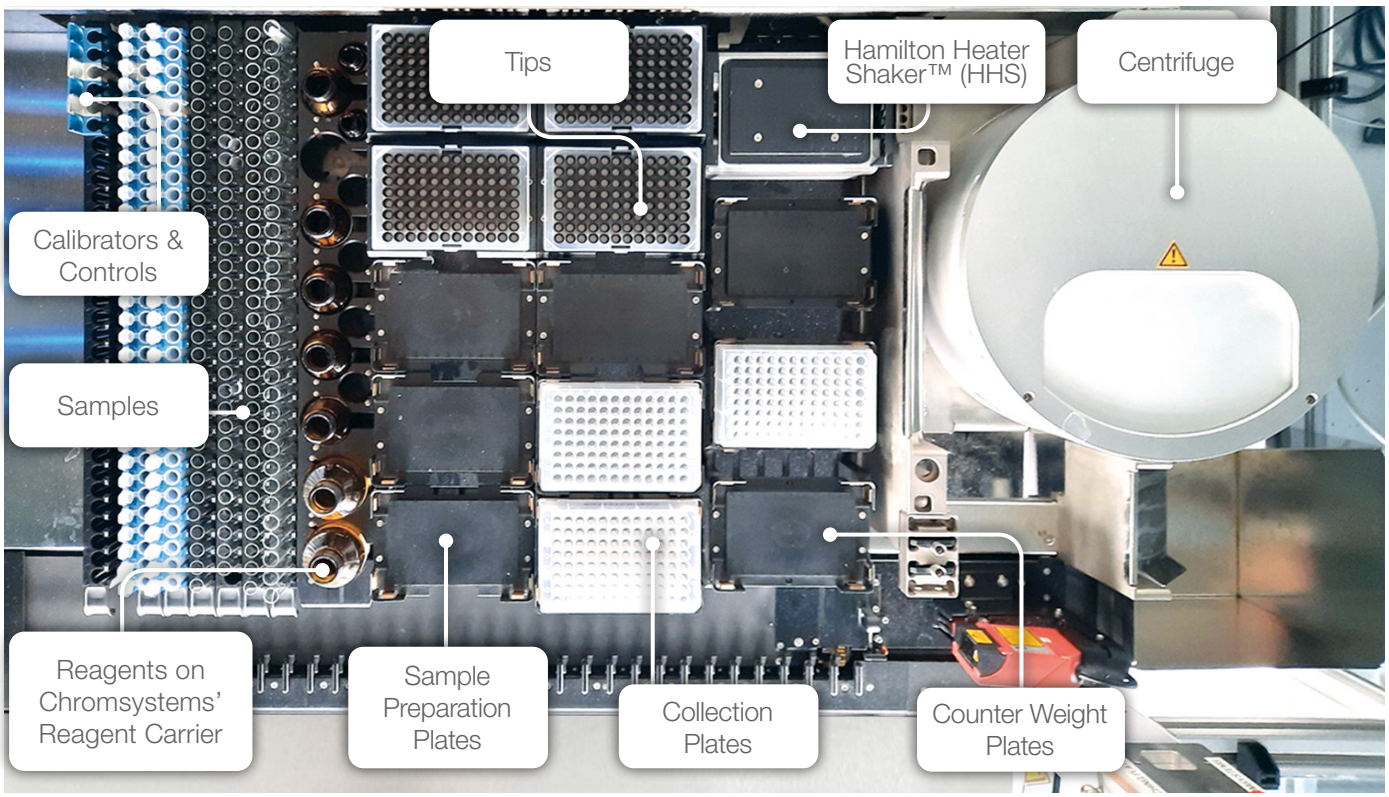

Figure 1: MassSTAR Deck Layout (optimized for Chromsystems). The deck layout for the MassTox® TDM Series A kit uses a Reagent Carrier for bottles instead of troughs.

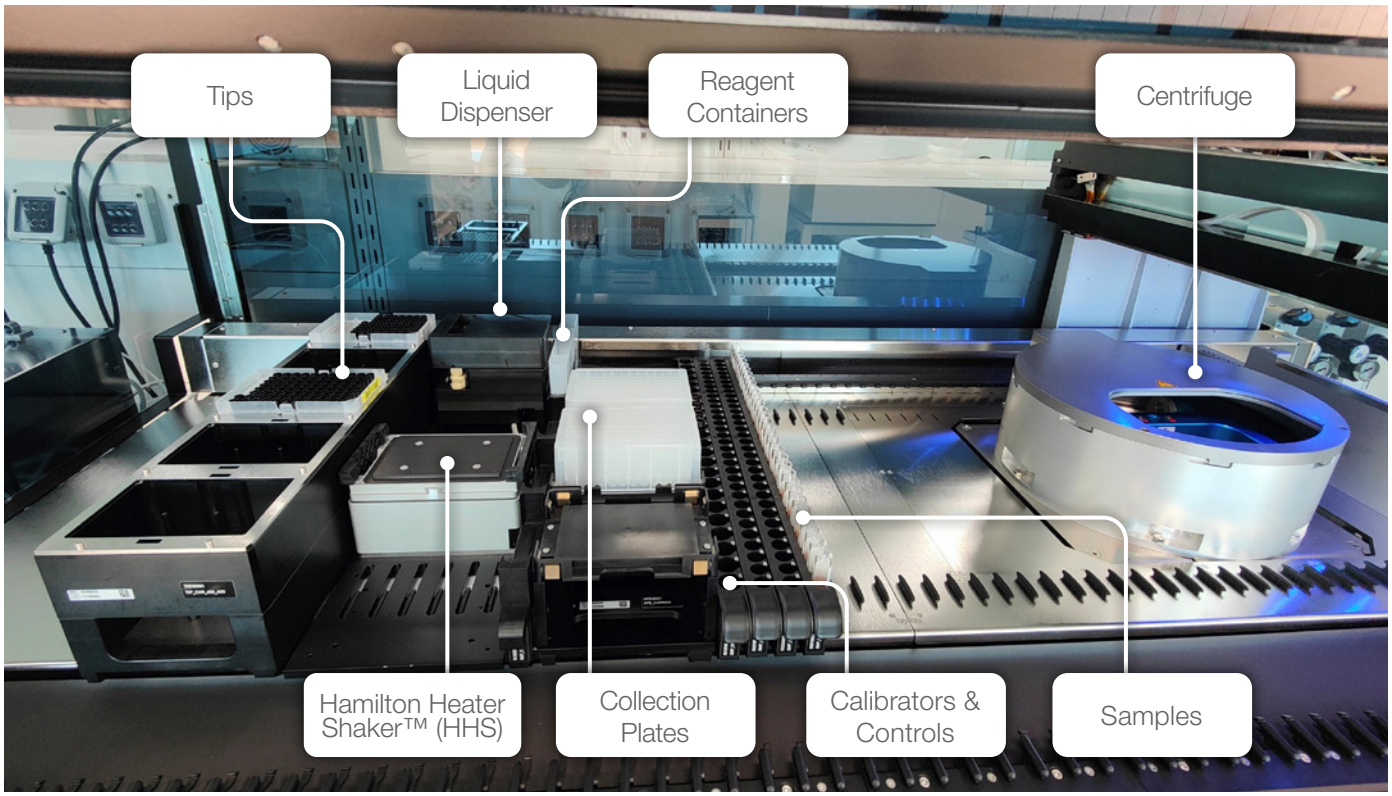

Figure 2: STAR CL Deck Layout (optimized for Shimadzu).

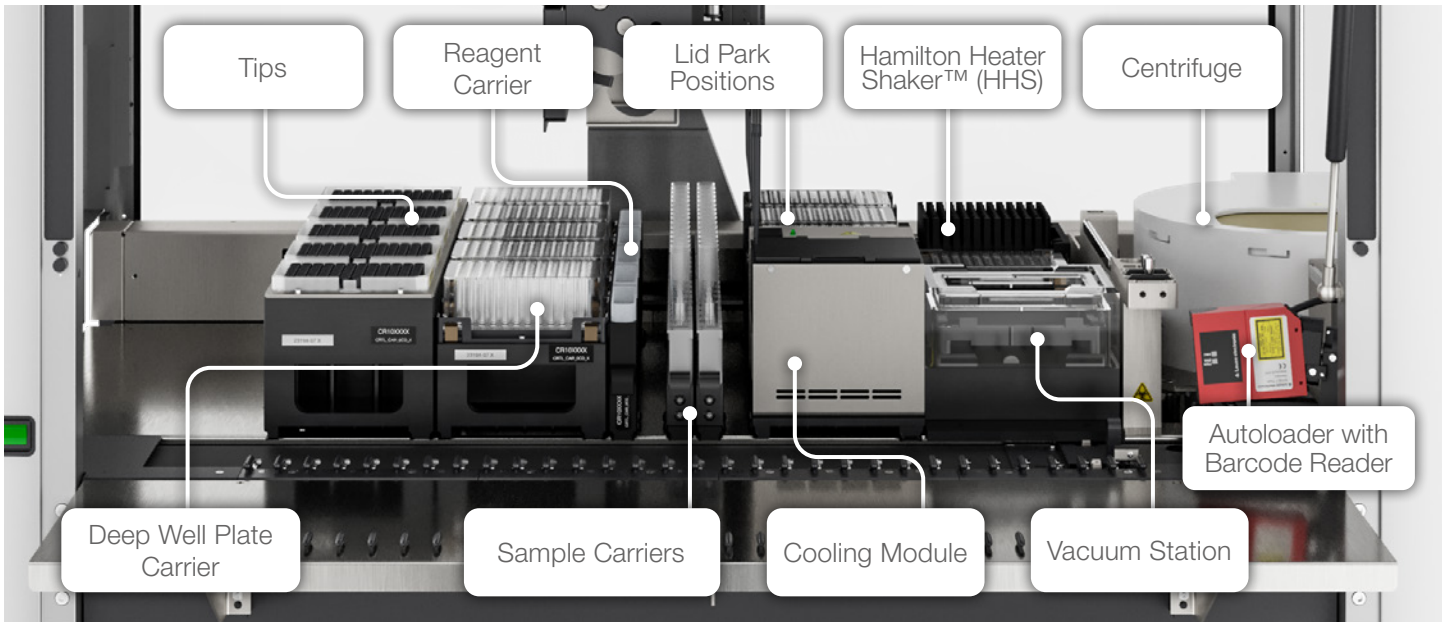

Figure 3: Dual MassSTAR, Deck Layout.

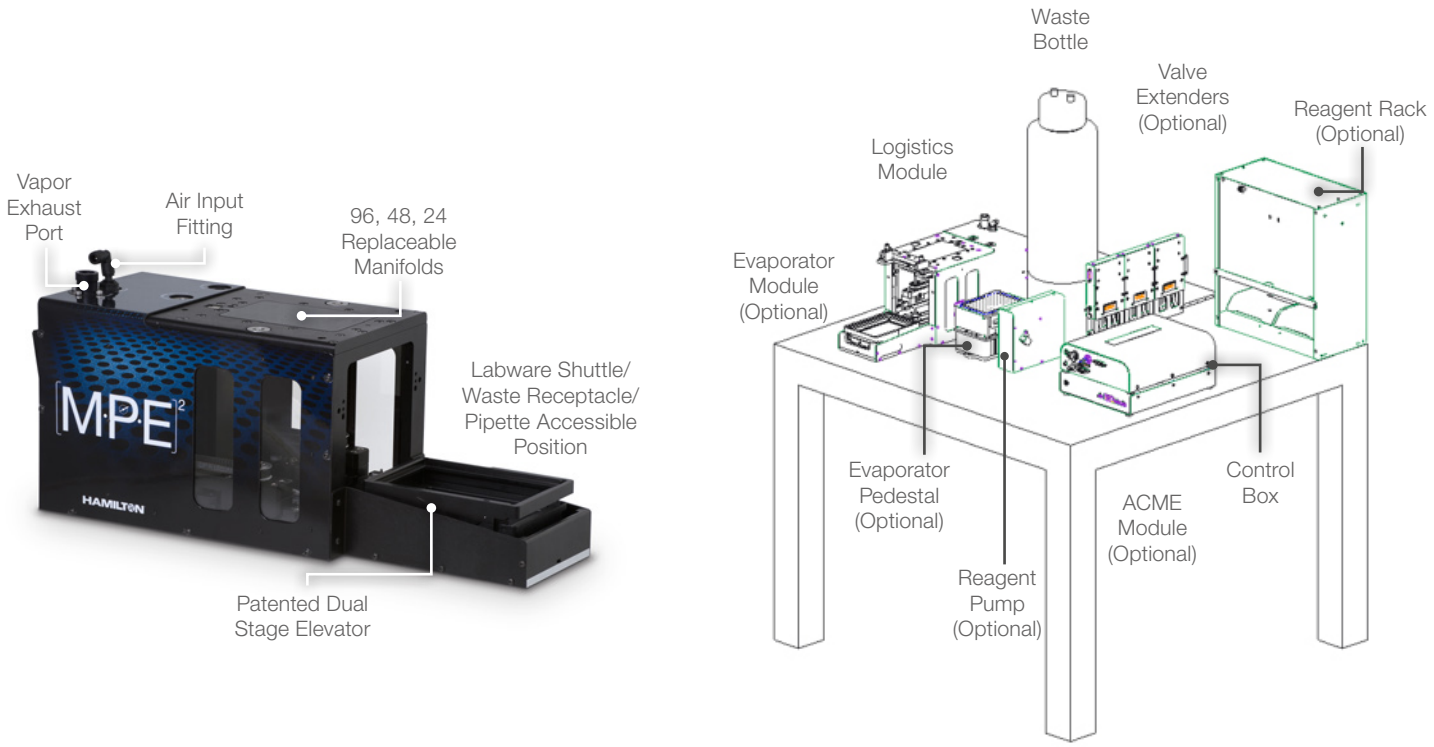

Figure 4: [MPE]²® Module. Left: Plate Logistics Module. Right: components of the full system.

microcentrifuges that allow full compatibility with most of the workflows for sample preparation.

For customers looking to process dried blood samples, we offer the easyPunch™ STARlet (**Figure 6**), a specialized platform for automated sample punching and processing. The easyPunch™ recognizes positively stained samples (e.g. blood on a white card) and negatively stained samples (e.g., saliva, plasma or urine on indicating cards) and makes punches of 1.2, 2, 3 and 6 mm directly into collection plates. The process is fully traceable.

The final decision about which instrument/ARW to use will depend on the specific needs of the customer.

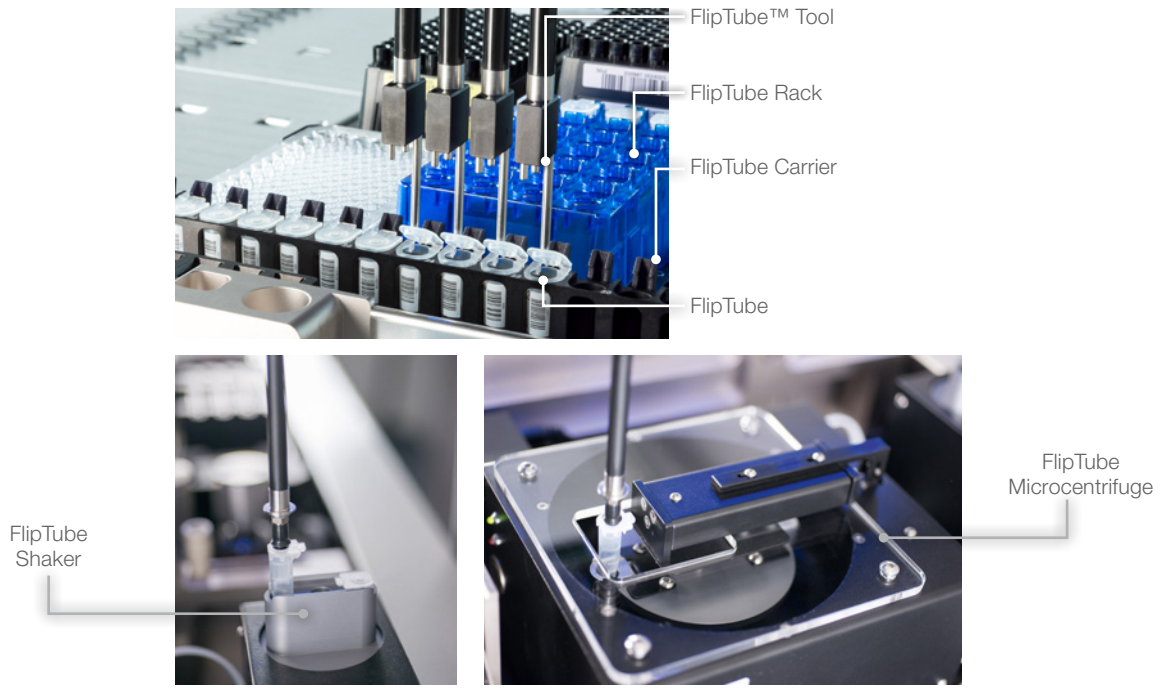

Figure 5: FlipTube's Solutions.

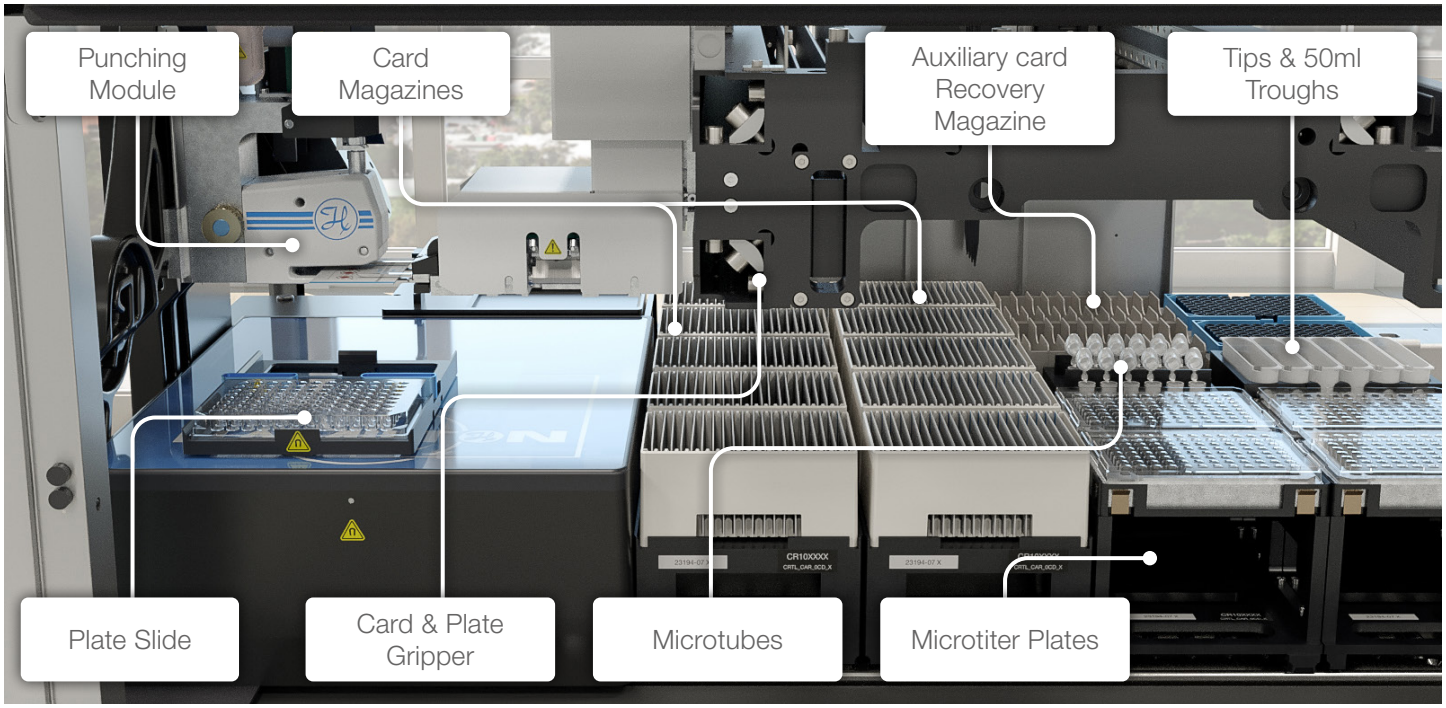

Figure 6: EasyPunch™ STARlet, Deck Layout.

Independent of the selection, all our solutions ensure:

Precision, accurate pipetting achieved through our various proprietary technologies:

1. Compression-Induced O-Ring Expansion (CO-RE®), for tip attachment and positioning
2. Liquid Level Detection (LLD), to detect the exact level of liquids in tubes or plates
3. Anti-Droplet Control (ADC), for correctly pipetting volatile organic solvents

Reproducibility, consistency ensured through repeated workflow testing.

Traceability, automated barcode verification of samples, reagents, plates and tips, as well as dynamic tracking of each aspiration and dispensation step using our Monitored Air Displacement (MAD) and Total Aspiration and Dispense Monitoring (TADM™) technologies.

APPLICATION NOTE

Automated Sample Preparation of More than 100 Drugs for Therapeutic Drug Monitoring

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Introduction

As each patient's absorption and metabolism of therapeutic drugs may vary, correct dosing is crucial to avoid toxic reactions and to keep the drug at therapeutic levels. Here is where Therapeutic Drug Monitoring (TDM) in serum or plasma patient samples comes into play. The gold standard for this analysis is LC-MS/MS; however, manual sample preparation can be a challenge when dealing with a high number of samples. Here, we describe the use of the Hamilton MassSTAR automation system together with ***Mass****Tox*® TDM Series A to perform automated sample preparation and analysis by LC-MS/MS of more than 100 drugs.

- Complete CE-IVD-certified workflow
- Easy-to-use

Figure 1. Hamilton MassSTAR

System Description

The MassSTAR (Figure 1) is based on a Hamilton Microlab® STARlet with four channels, a CO-RE® Gripper, a Barcode Reader and an integrated centrifuge. The deck consists of carriers for samples, reagents, calibrators and controls, two carriers for Deep-Well Plates (DWPs) and pipetting tips, as well as a Hamilton Heater Shaker (HHS) (Figure 2). Per run, up to 96 samples including calibrators and controls can be processed by the system. The method has been optimized to enable best performance.

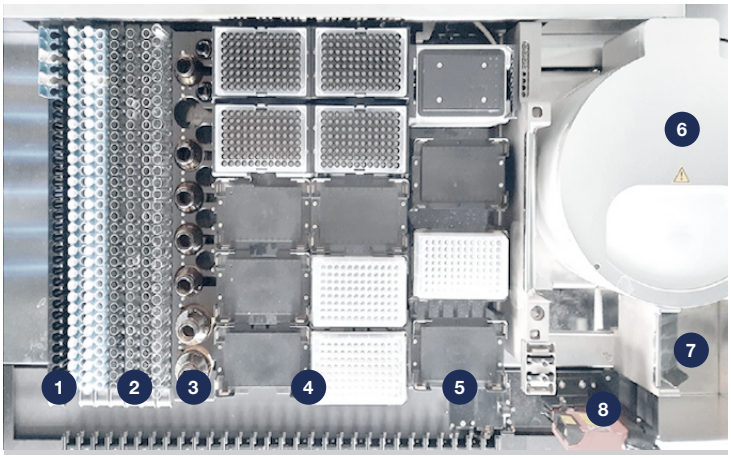

Figure 2. The Deck Layout of the MassSTAR for ***Mass****Tox*® **TDM Series A**

1. Carrier for calibrators and controls
2. Carriers for sample tubes
3. Chromsystems Reagent Carrier
4. Carrier for 96-well plates and tips
5. Carrier for HHS and counterweight plate
6. Centrifuge
7. Waste
8. Barcode Reader

Kit Description

The sample preparation is based on ***Mass****Tox*® TDM Series A from Chromsystems, a modular system that consists of three components: the specific parameter sets, the chromatographic column and the BASIC Kit A. The parameter sets contain individual calibrators, controls and internal standards for the specific drugs. The column itself allows the monitoring of nearly 200 parameters without column switching or change of the mobile phases. In combination with an almost identical sample preparation workflow throughout all parameter sets, this helps to minimize the workload in the laboratory. So far, sample preparation of more than 100 parameters can be automated on the MassSTAR, with more to follow. These include antidepressants, antiepileptic drugs, antimycotics, mycophenolic acid and neuroleptics (Test menu for ***Mass****Tox*® TDM Series A, see below). For a high data accuracy, nearly all parameters are safeguarded by internal standards. The traceability of results is assured by the use of matrix-based calibration material, which is again traceable to certified reference material.

Workflow

First, all resources are loaded and the barcode of samples and plates is traced. After homogenization, 50 µl of sample/calibrator/control are transferred to a 96 DWP. 25 µl of Extraction Buffer is added and shaken for 2 min at 600 rpm. 250 µl of prepared Internal Standard Solution are pipetted in each well and shaken for 30 s at 1000 rpm. The precipitate is then separated by centrifugation at 2000 x g for 5 min. The supernatant is transferred to a second DWP and diluted with Dilution Buffer. The dilution factor depends on the parameter set and the specific mass spectrometer.

Technology

One of the key safety features is the Total Aspiration and Dispense Monitoring (TADM™) of the instrument. Each aspiration and dispensing step in each pipetting channel is monitored in real-time. TADM™ verifies the sample transfer with a traceable digital audit trail and identifies errors such as blood clots in the sample or incorrect volumes that have been transferred. A Graphical User Interface (GUI) guides the user through the loading process along with an inventory check (e.g. barcode matching, checking if the plates are correctly positioned, verifying appropriate liquid levels for the reagents). This means that all reagents and consumables are available in sufficient quantities before the run is started, ensuring that no further user intervention is required, thereby providing additional walk-away time. Descriptive screens monitor the current state of the sample preparation process and show the steps the system will perform next. The GUI also prevents invalid user inputs and protects method files against unauthorized manipulation. Additionally, a tailor-made reagent carrier for ***Mass****Tox*® TDM Series enables the loading of assay reagents in their original containers. This means that the reagents do not have to be transferred, saving time, preventing contaminations and a mixing up of reagents, and decreasing the evaporation of the liquid.

Results

The recovery has been determined for all drugs and is dependent on the analyte, ranging from 81% to 115%.

The repeatability, intermediate precision and reproducibility have been measured to determine the overall method's precision. Samples of at least two different concentration levels were prepared ten times and measured in a single sequence. The variation coefficients ranged between 0.6 and 13.7%, depending on respective analytes and concentration level. Preparation and measurement of samples with at least three different concentration levels on different days (20 x 5 or 10 x 2 x 2 design) resulted in variation coefficients between 0.9 and 18.9%. Furthermore, the reproducibility was determined for at least three concentration levels in three different laboratories, on five different days with a sample preparation that was performed five times (3 x 5 x 5 design). The resulting variation coefficients were between 3.2 and 25.7%. Comprehensive data is available in the instruction manual for each parameter set.

The Lower Limit of Quantification (LLOQ) and Higher Limit of Quantification (ULOQ) have been determined by spiking serum and plasma samples with defined amounts of standard substances for all analytes. For all drugs, LLOQ and ULOQ cover the clinically relevant concentration range required for therapeutic drug monitoring.

Method comparison

Real patient samples have been collected and analyzed by using ***Mass****Tox*® TDM Series A. All samples have been processed manually and on the MassSTAR and compared.

Results show an excellent correlation and comparability between the sample preparation of the MassSTAR and manual processing (Examples see Figure 3).

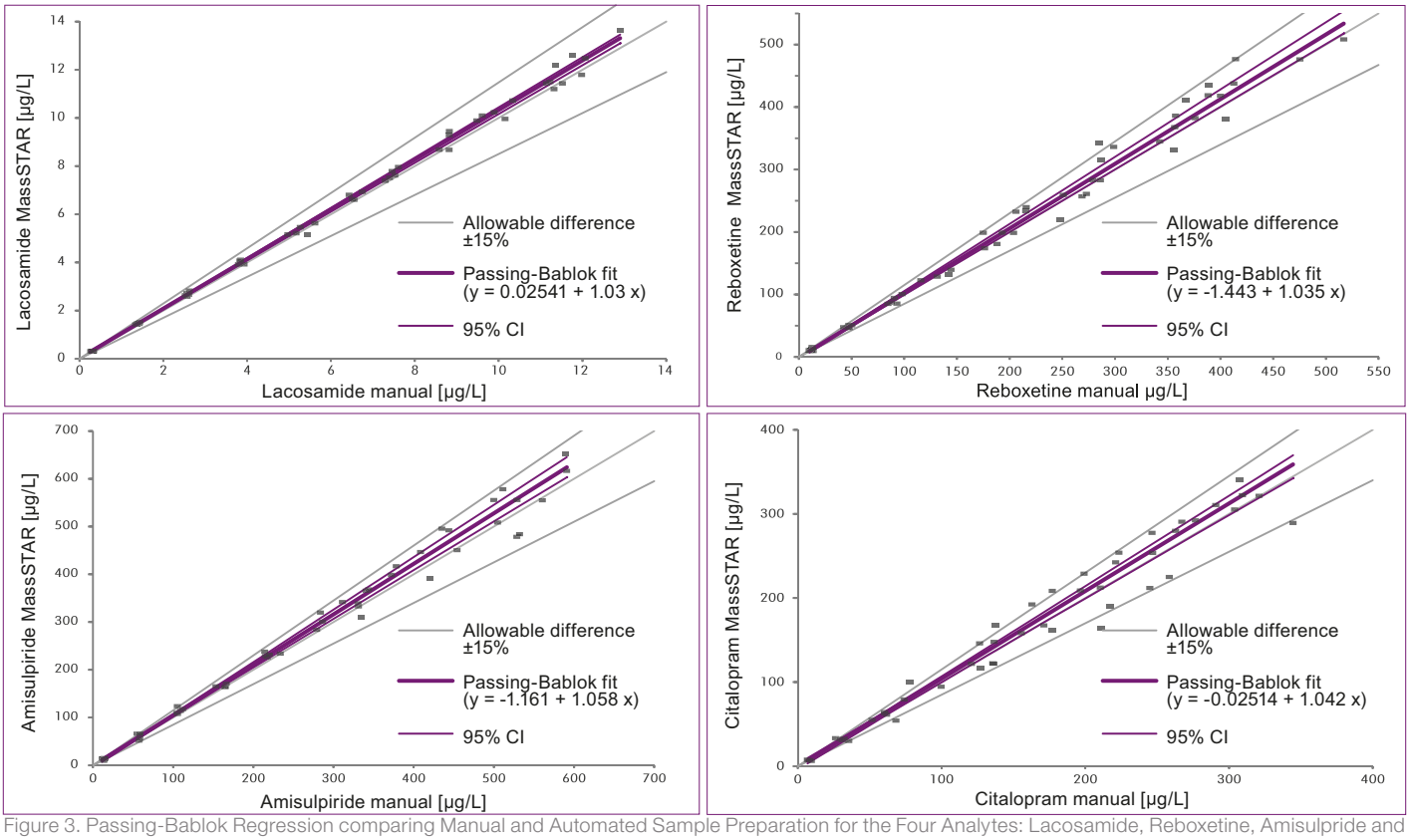

Figure 3. Passing-Bablok Regression comparing Manual and Automated Sample Preparation for the Four Analytes: Lacosamide, Reboxetine, Amisulpride and Citalopram. The graphs demonstrate a high correlation of data.

Others

Requirements	Part Number	Provider
MassSTAR	806170; CS806170	Hamilton Bonaduz AG; Chromsystems

The Complete Automated Testing Menu on the MassSTAR

*Mass*Tox® TDM Series A

Mass*Tox® TDM Series A Antidepressants 1/EXTENDED (order no. 92713/XT)*
Citalopram, N-Desmethylcitalopram, Duloxetine, Fluoxetine, Desmethylfluoxetine, Fluvoxamine, Mirtazapine, N-Desmethylmirtazapine, Paroxetine, Sertraline, N-Desmethylsertraline, Venlafaxine, O-Desmethylvenlafaxine

Mass*Tox® TDM Series A Antidepressants 2/Psychostimulants/EXTENDED (order no. 92715/XT)*
Atomoxetine, Bupropion, Erythro-Dihydrobupropion, Threo-Dihydrobupropion, Hydroxybupropion, Clomethiazole, Dosulepin, N-Desmethyldosulepin, Methylphenidate, Mianserin, Milnacipran, Moclobemide, Opipramol, Reboxetine, Ritalinic acid, Tianeptine, Tranylcypromine, Trazodone, Vilazodone, Vortioxetine

Mass*Tox® TDM Series A Antiepileptic Drugs All-in-One Method (order no. 92721/XT)*
Brivaracetam, Carbamazepine, Carbamazepine-10,11-epoxide, 10-OH-Carbamazepine, 10,11-Dihydroxycarbamazepine,N-Desmethylmesuximide, Ethosuximide, Felbamate, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, Oxcarbazepine, Perampanel, Phenobarbital, Phenylethylmalonamide (PEMA), Phenytoin, Pregabalin, Primidone, Retigabine, Rufi namide, Stiripentol, Sultiame, Theophylline, Tiagabine, Topiramate, Valproic acid, Vigabatrin, Zonisamide

Mass*Tox® TDM Series A Antimycotic Drugs (order no. 92722/XT)*
Anidulafungin, Caspofungin, Fluconazole, 5-Flucytosine, Isavuconazole, Itraconazole, Hydroxyitraconazole, Ketoconazole, Micafungin, Posaconazole, Voriconazole

Mass*Tox® TDM Series A Mycophenolic Acid (order no. 92716)*
Mycophenolic acid, Mycophenolic acid glucuronide

Mass*Tox® TDM Series A Neuroleptics 1/EXTENDED (order no. 92712/XT)*
Aripiprazole, Dehydroaripiprazole, Clozapine, N-Desmethylclozapine, Haloperidol, Olanzapine, N-Desmethylolanzapine, Quetiapine, Norquetiapine, Risperidone, 9-OH-Risperidone

Mass*Tox® TDM Series A Neuroleptics 2/EXTENDED 2 (order no. 92714/XT2)*
Amisulpride, Benperidol, Brexpiprazole, Bromperidol, Cariprazine, Chlorpromazine, Chlorprothixene, Flupentixol, Fluphenazine, Guanfacine, Iloperidone, Levomepromazine, Loxapine, Lurasidone, Melperone, Perazine, Perphenazine, Pimozide, Pipamperone, Promethazine, Prothipendyl, Sertindole, Sulforidazine, Sulpiride, Thioridazine, Ziprasidone, Zotepine, Zuclopenthixol

These products are not available in all countries. Please contact your local Chromsystems sales representative for availability in your region.

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APPLICATION NOTE

Automated Sample Preparation for Immunosuppressant Analysis

Authors: Maximilian Dietz[1], Martin Waterstraat[1] and Jan Feldmann[2]

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[2] Hamilton Bonaduz AG, Via Crusch 8, 7402 Bonaduz, Switzerland

Therapeutic Drug Monitoring (TDM) requires solutions that comply with IVD requirements and are easy-to-use. Chromsystems, together with Hamilton Robotics, has developed the MassSTAR (Figure 1), a CE-IVD-certified automated solution that will soon (2023) also be in-line with IVDR.

- Complete CE-IVD-certified workflow
- Easy-to-use

Figure 1. Hamilton MassSTAR

Introduction

Immunosuppressive drugs are used to prevent organ transplant rejection and to treat autoimmune diseases. As each patient's absorption and metabolism of the drugs varies, correct dosing is crucial to avoid toxic reactions while still keeping the drug at therapeutic levels, ensuring the patient's well-being.

TDM of the regularly prescribed immunosuppressive drugs cyclosporin A, everolimus, sirolimus and tacrolimus is common in clinical laboratories. The gold standard for the analysis of these drugs in whole blood is LC-MS/MS.

Whole blood samples tend to form blood clots, which is why their handling can be challenging. Process monitoring and high pipetting precision are crucial to ensure the quality and integrity of the results and compliance with regulatory requirements. However, current customized automation solutions tend to neglect these aspects.

System Description

The MassSTAR is based on a Hamilton Microlab® STARlet with four channels, a CO-RE® Gripper, a Barcode Reader and a centrifuge integrated to the right of the system. The deck consists of carriers for samples, one carrier for reagents and one for calibrators and controls. In addition, there are two carriers for Deep-Well Plates (DWPs), collection plates and pipetting tips as well as a carrier hosting a Hamilton Heater Shaker (HHS) and plates used as counter balances during centrifugation (Figure 2).

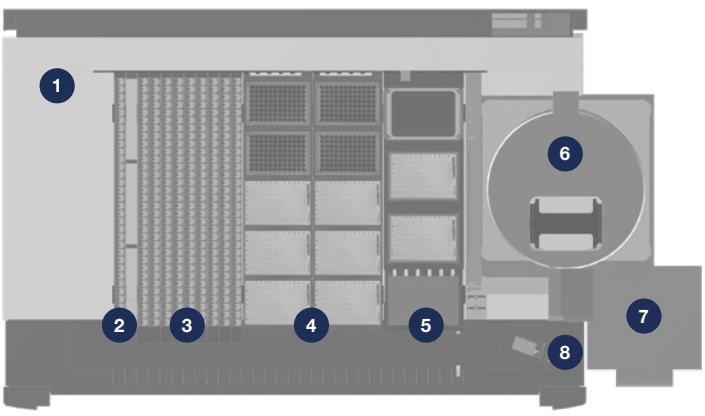

Figure 2. Deck Layout of the Hamilton MassSTAR

1. Carrier for calibrators and controls
2. Carrier for reagents (troughs)
3. Carriers for sample tubes
4. Carrier for 96-well plates and tips
5. Carrier for HHS and counterweight plate
6. Centrifuge
7. Waste
8. Barcode Reader

The system has a capacity of up to 176 samples per run, including controls and calibrators. The application is based on Hamilton's STAR IVD Software. The method has been optimized to enable best performance.

A user-friendly Graphical User Interface (GUI) makes the system easy-to-use and generates output files ready-to-use for all common LC-MS/MS systems.

CE-IVD-Certified Workflow

Kit Description

The Chromsystems ONEMINUTE ***Mass****Tox*® reagent kit for the analysis of immunosuppressants (Chromsystems PN 93900/1200/DWP) is used on this automation platform. It delivers robust, precise and reproducible results with a run time of approximately one minute per sample. The method is completely validated for the majority of tandem mass spectrometers on the market. Sample preparation is reduced to a simple and effective protein precipitation and online purification step (trap column) and, thus, reduces matrix effects drastically. Isotopically labeled internal standards compensate for all residual matrix effects. The use of multilevel calibrators (6PLUS1®) add to result accuracy.

Figure 3. Chromsystems ONEMINUTE ***Mass****Tox*® Immunosuppressant Reagent Kit

Workflow

First, all resources are loaded and the barcode of samples and plates are traced. Samples in Sarstedt 2.7 ml, 1.2 ml Monovette, Greiner 3 ml Vacuette or other tube types can be processed. They will then be mixed in a series of elaborate mixing steps to ensure perfect homogeneity before 50 µl of each sample is transfered to a dedicated DWP (Chromsystems PN 93956). Total Aspiration and Dispense Monitoring (TADM™) ensures proper sample pipetting. Samples that cannot be pipetted properly, e.g. samples with blood clots or short samples, will be recognized, excluded from further processing and flagged.

After sample transfer, 25 µl of internal standard and 100 µl of extraction reagent are added to the plate and agitated at 1,200 rpm for 2 min on the HHS. Afterward, 250 µl precipitation reagent is added to each sample, followed by another incubation step on the HHS for 3 min at 1,200 rpm. Finally, the system transports the plate to the centrifuge, where it is centrifuged for 3 min at 2,000 x g. The supernatant is then transferred via tip transfer into a collection plate (Chromsystems PN 93058). The samples are then ready to be analyzed by LC-MS/MS. For 96 samples, the entire process takes approximately 100 min.

Technology

Pipetting whole blood tends to be challenging as blood quickly settles down, reducing the homogeneity of the sample. In addition, blood tends to clot, which can generate problems during pipetting (e.g. by blocking tips and causing insufficient sample to be transferred), and lead to cross-contamination.

To ensure homogeneity of the sample and reduce clots, we use an elaborate mixing procedure, aspirating and dispensing the sample at different heights. This enables maximum homogeneity in the sample and dissolves most blood clots.

Immediately after mixing, we transfer the samples using TADM™. TADM™ monitors the pressure curve of the sample on the pipetting channel and recognizes when a transfer is out of the defined boundaries. An error will lead to a retry, and in the case of a repeated error, to the exclusion of the faulty sample (Figure 4).

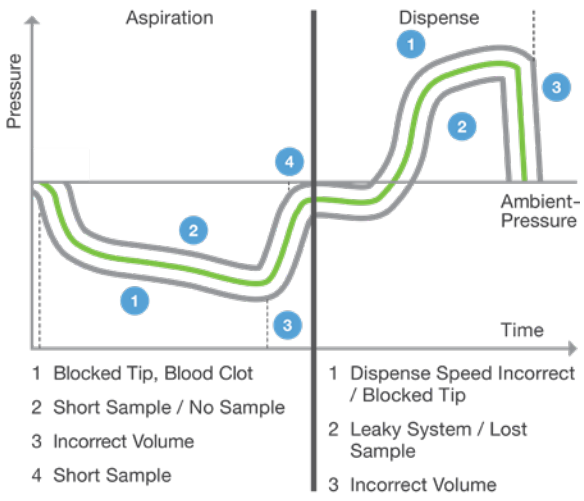

Figure 4. TADM™ Functionalities

Results

Repeatability & Within-Laboratory Precision

Performance data on repeatability and within-laboratory precision was determined by measuring three different samples and double processing on ten different days and two runs per day. The procedure is based on CLSI EP05-A3 and corresponds to a 10 x 2 x 2 test design (Table 1).

Table 1. Repeatability & within-Laboratory Precision

Substance	Sample	Mean [µg/l]	Repeatability		Within-laboratory precision	
			Coefficient of variation	95% confidence interval	Coefficient of variation	95% confidence interval
Cyclosporin A	Low	52.2	2.7%	2.1–3.9%	3.9%	3.0–5.7%
	Middle	263	3.1%	2.4–4.5%	3.9%	3.1–5.3%
	High	580	2.7%	2.1–3.9%	4.0%	3.0–5.8%
Everolimus	Low	2.17	5.8%	4.4–8.4%	6.0%	4.9–7.8%
	Middle	6.89	3.4%	2.6–4.9%	4.2%	3.4–5.5%
	High	15.8	3.0%	2.3–4.3%	3.4%	2.7–4.4%
Sirolimus	Low	2.80	3.8%	2.9–5.5%	4.9%	4.0–6.4%
	Middle	8.11	4.0%	3.0–5.7%	4.4%	3.6–5.7%
	High	19.1	3.0%	2.3–4.4%	3.3%	2.7–4.3%
Tacrolimus	Low	2.87	4.3%	3.3–6.3%	5.3%	4.3–7.1%
	Middle	9.40	3.4%	2.6–5.0%	3.8%	3.1–4.9%
	High	20.6	2.6%	2.0–3.7%	3.1%	2.5–4.1%

Reproducibility

The performance data on reproducibility was determined at three sites on the basis of three different samples by 5-fold processing on five different days. The procedure is based on CLSI EP05-A3 and corresponds to a 3 x 5 x 5 test design (Table 2).

Table 2. Reproducibility

Substance	Sample	Mean [µg/l]	Reproducibility	
			Coefficient of variation	95% confidence interval
Cyclosporin A	Low	49.9	5.2%	3.9–7.7%
	Middle	258	4.3%	3.5–5.7%
	High	581	5.0%	3.4–9.7%
Everolimus	Low	2.19	12.8%	9.9–18.3%
	Middle	6.74	6.3%	5.2–7.9%
	High	15.5	6.2%	5.1–7.9%
Sirolimus	Low	2.79	7.1%	5.8–9.4%
	Middle	8.04	5.8%	4.8–7.4%
	High	18.8	5.8%	4.8–7.2%
Tacrolimus	Low	2.84	8.0%	6.4–10.6%
	Middle	9.14	5.8%	4.5–8.1%
	High	20.3	4.5%	3.7–5.8%

MassSTAR vs. Reference Method

The automated method's results with the MassSTAR were compared with a published reference method [1]. The results of the Passing-Bablok analysis show that the sample preparation on MassSTAR is comparable to the reference method. Over the entire concentration range, the slope was between 0.95 to 1.05 for all four analytes, cyclosporin A, everolimus, sirolimus and tacrolimus (Figure 5).

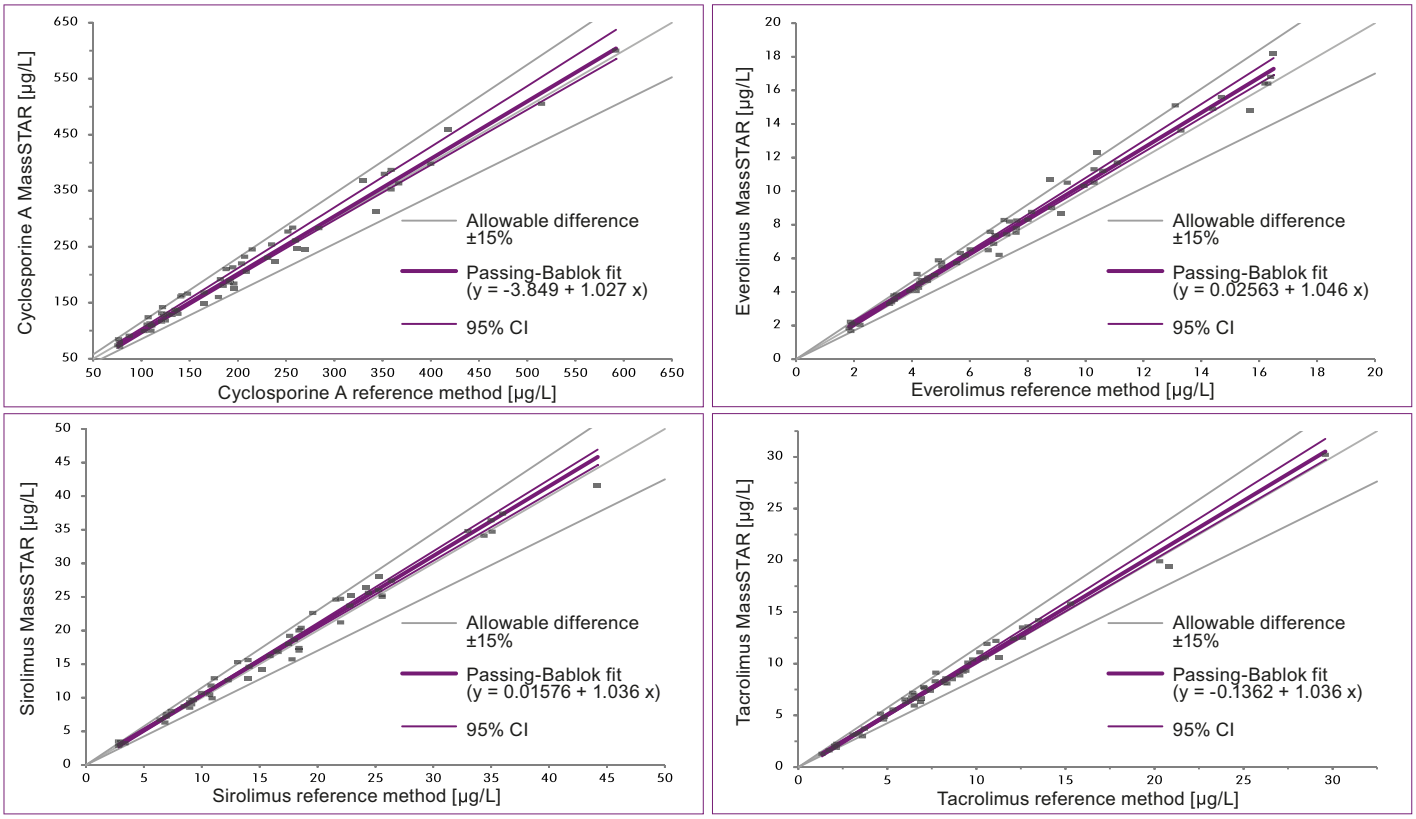

Figure 5: Passing-Bablok Analysis of the Automated Sample Preparation using MassSTAR vs. the Reference Method for Cyclosporin A, Everolimus, Sirolimus and Tacrolimus.

Others

Requirements	Part Number	Provider
MassSTAR	806170; CS806170	Hamilton Bonaduz AG; Chromsystems
Mass*Tox*® Immunosuppressants in whole blood ONEMINUTE test	93900/1200/DWP	Chromsystems

[1] Taibon J, van Rooij M, Schmid R, Singh N, Albrecht E, Anne Wright J, Geletneky C, Schuster C, Mörlein S, Vogeser M, Seger C, Pongratz S, Kobold U. An isotope dilution LC-MS/MS based candidate reference method for the quantification of cyclosporine A, tacrolimus, sirolimus and everolimus in human whole blood. Clinical Biochemistry 2020;82:73–84.

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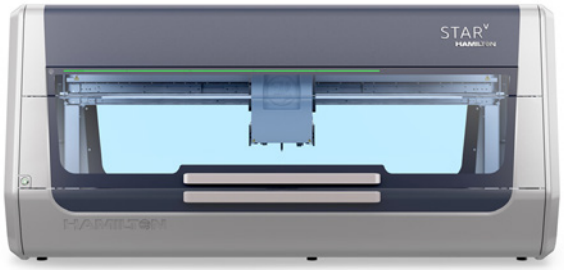

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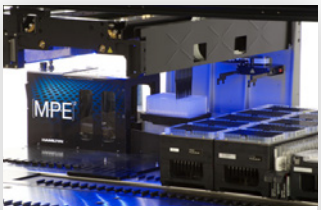

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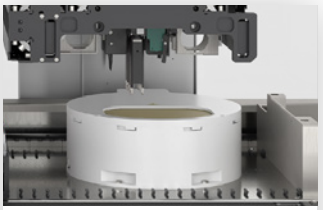


Centrifuge

For spinning samples up to 4,200 rpm

ODTC

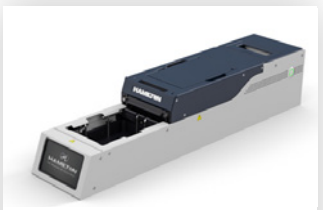
For incubations between +4°C and +99°C

Punching Module

For card punching

ApH Module

For pH measurement



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For accessing samples in various sealed labware

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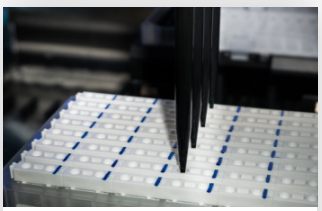

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