APPLICATION NOTE

Automated Sample Preparation for Immunosuppressant Analysis

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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).

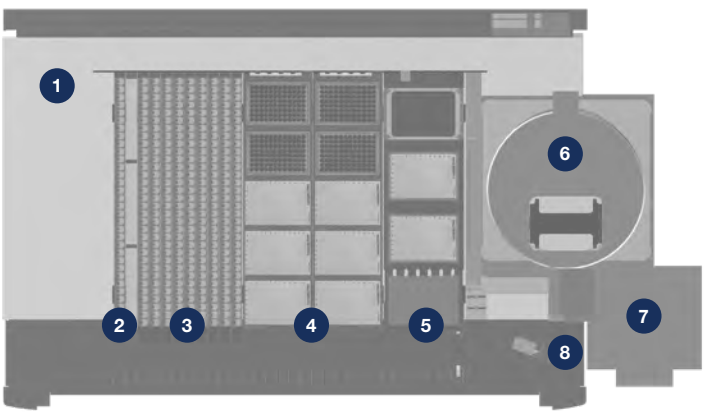

Figure 2. Deck Layout of the Hamilton MassSTAR

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).

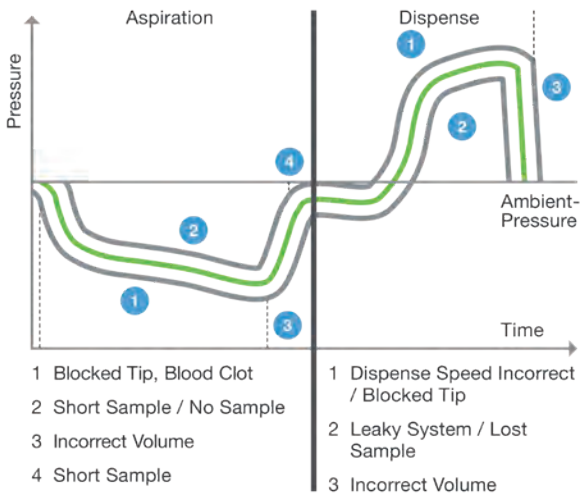

Figure 4. TADM™ Functionalities

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).

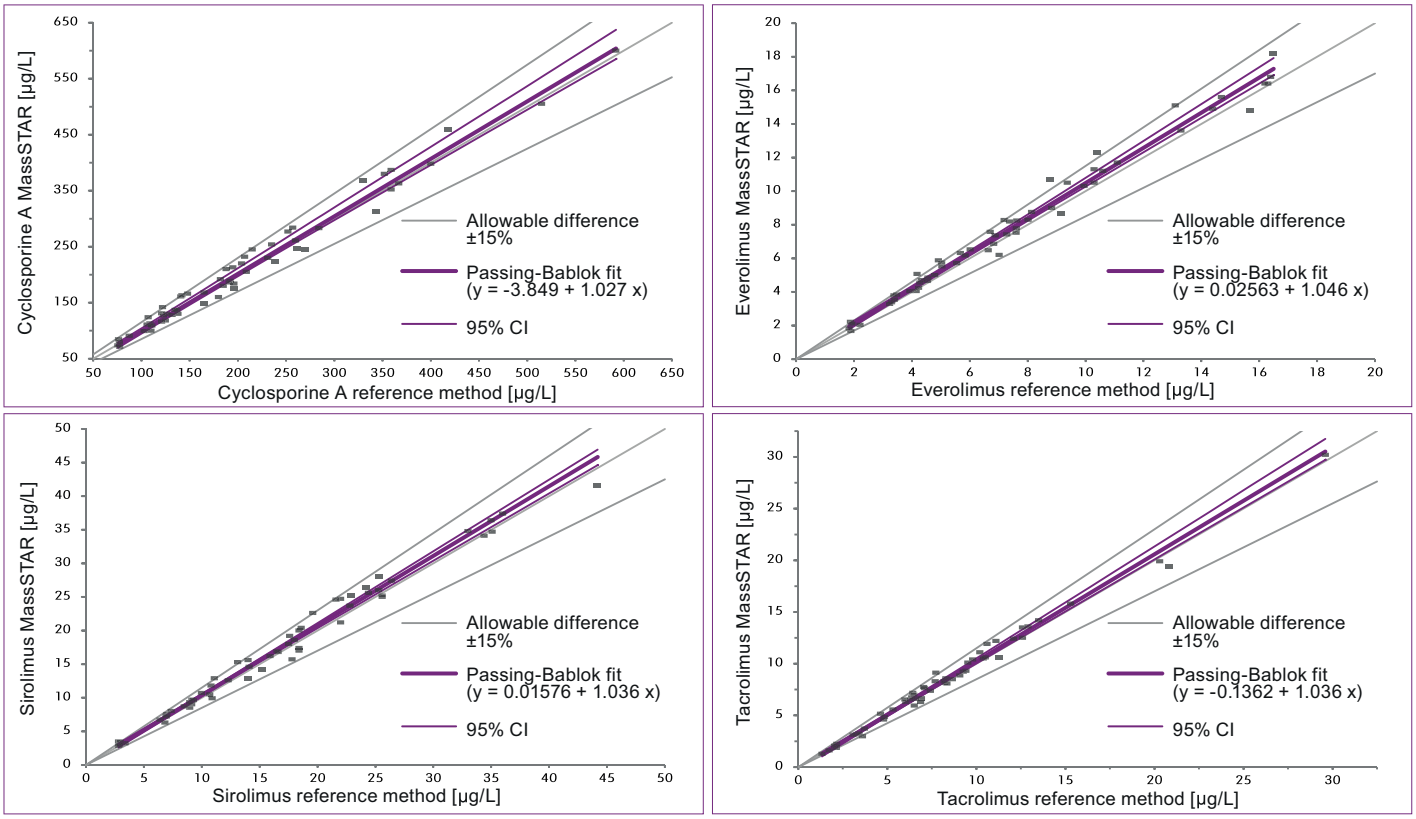

Figure 5: Passing-Bablok Analysis of the Automated Sample Preparation using MassSTAR vs. the Reference Method for Cyclosporin A, Everolimus, Sirolimus and Tacrolimus.

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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