Automated Sample Preparation for Immunosuppressant Analysis

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Therapeutic drug monitoring requires solutions that comply with IVD guidelines, enable a full audit trail and are easy to use. Chromsystems together with Hamilton has developed MassSTAR as a solution that meets these requirements.

- Complete CE-IVD certified workflow
- Easy to use
- Full audit trail

***Figure 1**. Hamilton MassSTAR*

Introduction

Immunosuppressive drugs are applied to prevent the body from rejecting an organ transplant or to treat autoimmune diseases. As each patient's absorption and metabolism of the drugs varies, correct dosing is crucial to avoid toxic reactions but still keep the drug at effective levels and therefore ensure the wellbeing of the patient.

Therapeutic drug monitoring of the regularly prescribed immunosuppressive drugs, cyclosporin A, everolimus, sirolimus and tacrolimus, is common in clinical laboratories. The gold standard for analysis of those drugs is LC-MS/MS.

One of the challenges laboratories have been facing is the sample preparation. Monitoring of the processing and a full audit trail are crucial to ensure quality and integrity of results. Current customized automation solutions focus on the processing part but tend to neglect the monitoring and audit trail.

***Figure 2**. The deck layout of the MassSTAR*

System Description

MassSTAR is based on a Hamilton Microlab STARlet IVD with 4 channels, CO-RE Gripper and centrifuge integration 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 filter plates, collection plates and pipetting tips as well as a Hamilton Heater Shaker used for incubation. The system has a capacity of up to 288 samples per run including controls and calibrators.

The application software is based on Hamilton's Vector 4.3 IVD. The method has been optimized to enable best performance. A user friendly GUI makes the system easy to use and generates output files ready to use for all common LC-MS/MS system.

CHROMSYSTEMS®

HAMILTON®

CE-IVD validated optimized workflow

Kit Description

The Chromsystems **ONE*MINUTE* *Mass*Tox®** reagent kit for the analysis of immunosuppressants (Chromsystems PN 93900) is used on this automation platform. It delivers robust, precise and reproducible results with a run time of approximately one minute per sample, sufficient instrument sensitivity provided. The method is completely validated for the majority of tandem mass spectrometer 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 labelled internal standards compensate all residual matrix effects. The use of multilevel calibrators (**6PLUS1®**) adds to result accuracy.

***Figure 3**. Chromsystems **ONE*MINUTE* *Mass*Tox® immunosuppressant reagent kit*

Workflow

First all resources are loaded and the barcode of samples and plates is traced. Samples in Sarstedt 2.7 ml or 1.2 ml Monovette or Greiner 3 ml Vacuette can be processed. They will then be mixed in a series of elaborate mixing steps to ensure perfect homogenity of the samples directly before 50 µl of each sample is transfered to a dedicated deep well filter plate (Chromsystems PN 93957). Total Aspiration and Dispense Monitoring (TADM) is used to ensure proper pipetting of samples. 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 internal standard and 100 µl extraction reagent is added and the filter plate agitated at 1200 rpm for 2 min on a heater shaker. Afterwards, 250 µl precipitation reagent is added to each sample, followed by another incubation step on the heater shaker for 3 min at 1200 rpm. Finally, the system transports the plate to the centrifuge where it is filtered into a collection plate (Chromsystems PN 93058) for 3 min at 2000 g. The samples are then ready to be analyzed by LC-MS/MS. For 96 samples, the whole process takes about 90 min.

Technology

Pipetting whole blood tends to be challenging as blood will quickly settle down and therefore reduce homogeneity of the sample. In addition, blood tends to clotting and this blood clots can generate problems during pipetting e.g. by blocking tips and causing insufficient sample to be transferred. In addition, clots transported in tips could lead to cross contamination of other samples.

To ensure homogeneity of the sample and reduce clots, we are using 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 Total Aspiration and Dispense Monitoring (TADM). TADM monitors the pressure curve of the sample pipetting and recognizes when a transfer is out of the defined boundaries. This can be caused by a number of different reasons. Identification of an error will thus immediately lead to identification and, after one retry, exclusion of all faulty samples.

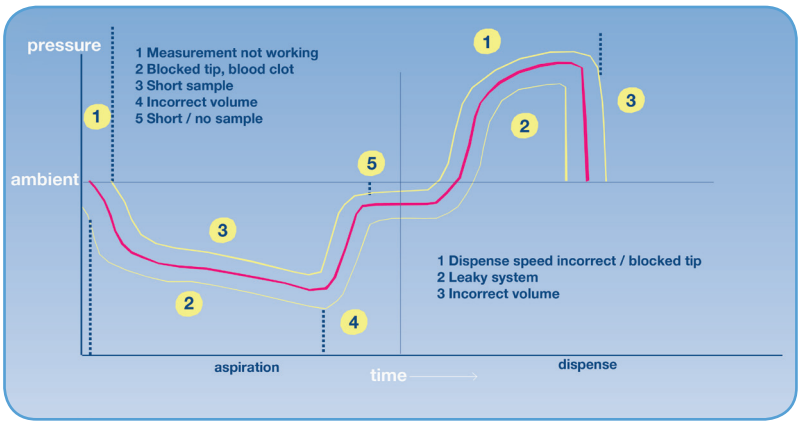

***Figure 4.** TADM functionalities*

Results

Blood samples have been spiked with defined amounts of pure analyte. The spiked samples have been processed independently several times using the automated optimized protocol for Chromsystems' ***Mass**Tox*®** Immunosuppressant kits on the Hamilton MassSTAR. The samples have been measured using an AB Sciex API 4500 mass spectrometer.

Recovery rate

Recovery rate of spiked analyte has been measured by the slopes of calibration curves and dilutions of standard solutions. All recovery rates are close to 100 %.

Substance	Recovery rate (%)
Cyclosporin A	98
Everolimus	100
Sirolimus	104
Tacrolimus	93

***Table 1**. Recovery rate*

Linearity

Linearity has been determined by spiking whole blood samples with defined amounts of standard substance. The lower limit of quantification (LOQ) has been measured by dilution of standard substance with calibrator 1 (Chromsystems PN 28039/1) with blank matrix. Linearity for cyclosporin ranges from LLOQ 6 µg/l to minimum 2000 µg/l. For everolimus, sirolimus and tacrolimus, linearity ranges from 0.5 µg/l to min 100 µg/l, respectively.

Analyte	LLOQ (µg/l)	Linear area until min. (µg/l)
Cyclosporin A	6	2000
Everolimus	0.5	100
Sirolimus	0.5	100
Tacrolimus	0.5	100

***Table 2**. Linearity*

Intra-assay reproducibility

The variation coefficient has been determined at 4 different concentrations by multiple processing of the same samples in one assay (n=10). The variation coefficient has always been < 7 %.

Analyte	Variation coefficient in % (at concentration in µg/l)			
Cyclosporin A	3.6 (49.7)	5.3 (249)	5.7 (484)	6.0 (1116)
Everolimus	5.2 (2.7)	6.9 (4.8)	5.0 (9.4)	2.7 (33.1)
Sirolimus	5.6 (2.7)	5.7 (9.7)	5.7 (18.5)	2.8 (37.6)
Tacrolimus	4.5 (2.7)	5.6 (7.4)	1.8 (15.3)	2.5 (32.2)

***Table 3**. Intra-assay reproducibility*

Inter-assay reproducibility

Interassay reproducibility has been determined at 4 different concentrations by multiple processings of the same samples at separate days (n=10). The variation coefficient has always been significantly < 10 %.

Analyte	Variation coefficient in % (at concentration in µg/l)			
Cyclosporin A	6.8 (49.7)	7.8 (249)	7.7 (484)	6.6 (1116)
Everolimus	9.5 (2.7)	8.5 (4.8)	8.8 (9.4)	6.6 (33.1)
Sirolimus	9.4 (2.7)	5.8 (9.7)	6.8 (18.5)	5.4 (37.6)
Tacrolimus	7.0 (2.7)	6.8 (7.4)	5.5 (15.3)	5.4 (32.2)

***Table 4**. Inter-assay reproducibility*

Manual vs Automated Processing

Real clinical whole blood samples have been collected and have been subject of processing using Chromsystems' ***Mass**Tox*®** Immunosuppressant kit. All samples have been processed manually as well as on the MassSTAR.

Results show that the sample preparation on MassSTAR is comparable to manual with a coefficient of determination (R^2) significantly >0.95 for all 4 analytes of interest, cyclosporin A, everolimus, sirolimus and tacrolimus (Figure 5).

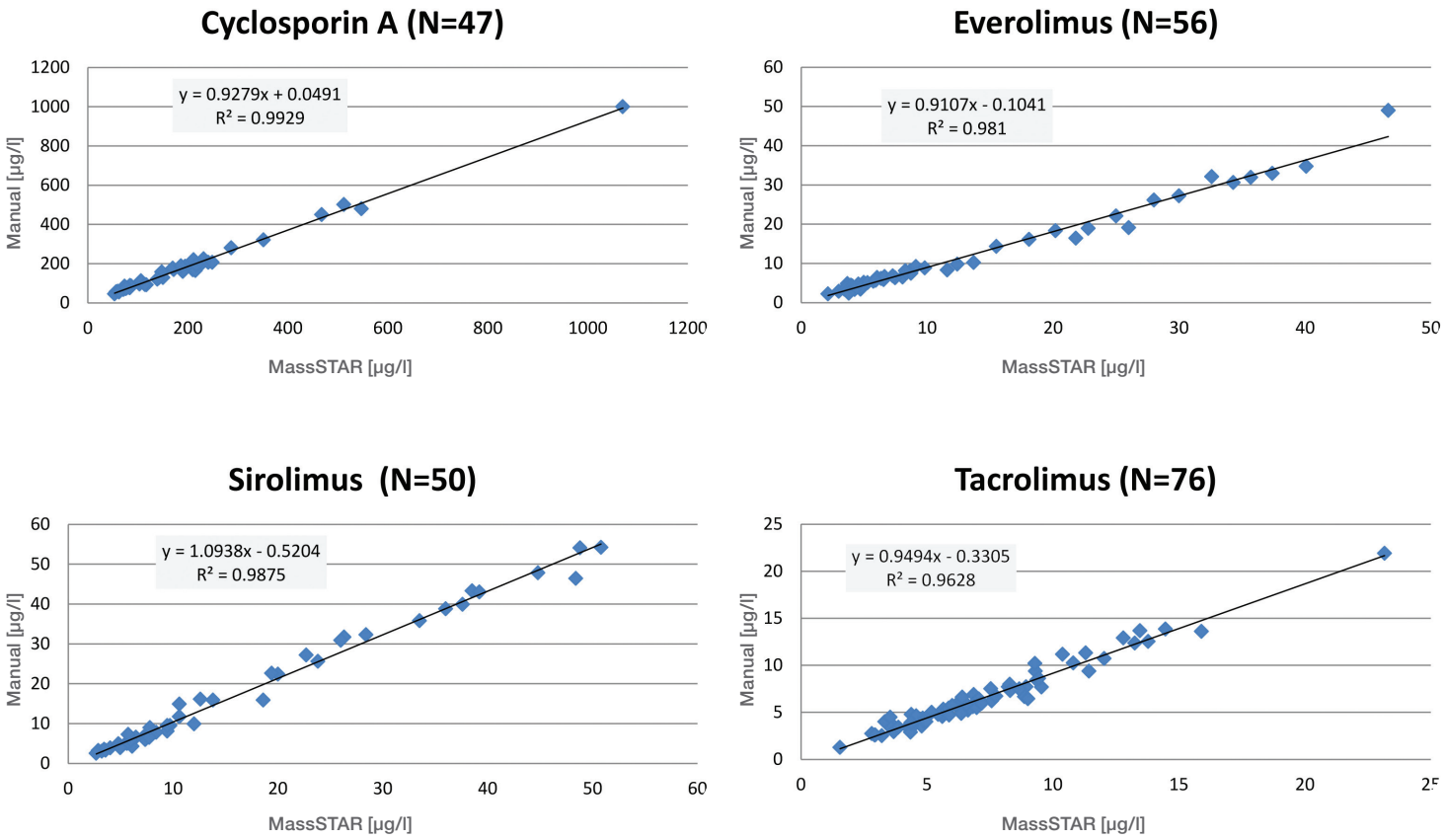

Figure 5. *Regression of manual sample preparation vs automated sample preparation using MassSTAR for Cyclosporin A, Everolimus, Sirolimus and Tacrolimus*

Hamilton Hardware	
MassSTAR	PN806170
Upgrade kit Chromsystems ***Mass**Tox*® Immunosuppressants	PN806173

Chromsystems reagent kit	
***Mass**Tox*® Immunosuppressants	PN 93900 / Chromsystems

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