From venous blood to VAMS – adaptation of a commercial CE-IVD assay kit for the determination of Tacrolimus, Sirolimus, Everolimus and Cyclosporin A.

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BACKGROUND

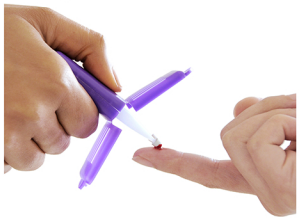
TDM of immunosuppressants is extremely important topic, especially when it comes to sample collection at home coupled with simple and fast sample preparation and analysis. The study is focused on the check if optimization of ready-to-use kit (Chromsystems CE-IVD kit: 93000) for determination of Tacrolimus (TAC), Sirolimus (SIR), Everolimus (EVR) and Cyclosporin A (CSA) in venous blood can be successfully applied for VAMS-collected blood and preliminary venous blood to Mitra® (VB/M) correlation study.

METHODS

Venous blood (Chromystems porcedure)

- Pipette 50 µL of sample/calibrator/Mass-Check® into a 1.5 mL tube.
- Add 25 µl of the reconstituted ISTD solution.
- Add 100 µl of extraction buffer, vortex for 10 s and incubate for 2 minutes at 20-25 °C.
- Add 250 µl of precipitating reagent, vortex for 1 minute and incubate for 2 minutes at 20-25 °C.
- Centrifuge for 5 minutes at 15000 x g.
- Transfer the supernatant to the vial.
- Inject 2-5 µl (SCIEX 5500+ QTRAP, Exion AC LC-MS/MS).

Mitra® - optimized porcedure based on Chromsystems kit



- Pipette min. 40 µL sample/calibrator/Mass-Check® on petri dish.
- Collect the material on the Mitra® sampler (10 µl).
- Dry samples at least 2h in the dark, 20-25°C.
- Transfer the Mitra® sampler to a 2 mL tube.
- Prepare an extraction buffer containing ISTD (20 ul ISTD + 100 ul extraction buffer x number of samples + 1)
- Add 120 µl of extraction buffer containing ISTD.
- Vortex for 10 s and then sonicate for 5 min at 20-25 °C.
- Add 80 µl of precipitating reagent, vortex for 10 s and sonicate for 5 min at 20-25 °C.
- Centrifuge for 5 minutes at 15000 x g.
- Transfer 100 µl of supernatant to the insert (100 µl on the feet) placed in the vial.
- Inject 10 µl (SCIEX 5500+ QTRAP, Exion AC LC-MS/MS).

Q1	Q3	Dwell time [ms]	Id	DP	EP	CE	CXP
821.5	768.5	50	Tacrolimus	110	10	30	12
824.5	771.5	50	Tacrolimus 13CD2	110	10	30	12
931.6	864.5	50	Sirolimus	110	10	25	14
934.6	864.5	50	Sirolimus-D3	110	10	25	14
975.6	908.6	50	Everolimus	110	10	27	14
979.6	912.6	50	Everolimus-D4	110	10	27	14
1219.9	1202.8	50	Cyclosporin A	110	10	26	16
1223.9	1206.8	50	Cyclosporin A-D4	110	10	26	16

IS Parameters	
CUR	30
CAD	9
IS	5500
TEM	300
GS1	45
GS2	45

Data processing, quantitation and basic statistical analysis was done in SciexOS 3.3 software (SCIEX).

RESULTS

Tab. 1. Validation results for collected on 10 µl Mitra® sampler lowest analytes concetrations provided with the kit (CAL 1, 4 replicates). LOD was not determined, however additional analyses showed that lower that 50-100 pg/ml concentration for each compound can be achieved.

Compound	LLOQ				ULOQ [ng/ml]	Linearity [R ≥ 0.995]
	C [ng/ml]	S/N ≥ 10	Accuracy [100±15%]	%CV [±15%]		
TAC	1.28	11.66	102.75	12.1	37.50	0.99907
SIR	2.10	15.15	100.05	12.3	43.40	0.99872
EVR	2.20	14.82	100.95	8.7	42.25	0.99826
CSA	24.20	52.68	111.30	7.9	772.00	0.99928

Signal-to-noise (S/N) was determiend with peak-to-peak algorithm and averged from 4 replicates.

Tab. 2. Results of preliminary study for VB/M correlation included 15 patients per compound where venous blood and 10 µl Mitra® samples were collected at the same time. No patients for EVR were tested during the study.

TAC

Sample	Venous blood [ng/ml]	Mitra [ng/ml]	VB/M
1	4.011	5.257	0.763
2	9.085	7.686	1.182
3	6.65	12.425	0.535
4	5.198	8.861	0.587
5	7.579	9.945	0.762
6	5.794	9.437	0.614
7	8.577	12.718	0.674
8	4.491	4.176	1.075
9	12.77	17.11	0.746
10	8.794	11.648	0.755
11	10.107	14.921	0.677
12	7.155	9.388	0.762
13	8.329	13.555	0.614
14	7.353	9.699	0.758
15	10.103	13.573	0.744
		Mean	0.750
		SD	0.172
		%CV	22.923

SIR

Sample	Venous blood [ng/ml]	Mitra [ng/ml]	VB/M
157	2.196	2.771	0.792
158	3.798	3.026	1.255
159	1.089	1.867	0.583
160	3.763	5.619	0.670
161	2.212	3.144	0.704
162	5.853	6.257	0.935
163	6.43	4.973	1.293
164	0.395	1.705	0.232
165	2.532	2.717	0.932
166	4.739	5.78	0.820
167	4.141	6.733	0.615
168	5.758	7.456	0.772
169	0.316	0.319	0.991
170	0.383	0.858	0.446
171	7.887	12.687	0.622
		Mean	0.777
		SD	0.281
		%CV	36.105

CSA

Sample	Venous blood [ng/ml]	Mitra [ng/ml]	VB/M
187	56.379	44.22	1.275
188	92.872	78.181	1.188
189	81.334	71.476	1.138
190	175.848	174.666	1.007
191	181.693	136.31	1.333
192	79.848	63.049	1.266
193	240.989	145.212	1.660
194	185.607	176.692	1.050
195	126.013	110.683	1.139
196	102.756	118.139	0.870
197	167.541	180.644	0.927
198	71.611	56.052	1.278
199	521.659	456.524	1.143
200	327.406	439.069	0.746
201	123.286	99.666	1.237
		Mean	1.150
		SD	0.219
		%CV	19.014

CONCLUSIONS

Optimized for 10 µl Mitra® sampler procedure met the validation criteria with the use of Chromsystems kit reagents and materials.

Additional steps required for Mitra® sample preparation are simple, robust and automation friendly.

The VB/M correlations revealed that the capillary blood concentration of TAC and SIR are lower than in venous blood contrary to concentration of CSA. This data may support the fact that TAC and SIR enters erythrocytes in about 90%, while CSA only in 50% and the capillary blood has higher amount of serum fraction. However, due to relatively small number of patients included in the study it needs further investigation on a larger group of patients and deeper statistical analysis