Maximizing accuracy with LC-MS/MS immunosuppressant drugs assay

Ready to use testing panel for cyclosporin A, everolimus, sirolimus and tacrolimus

Immunosuppressant drugs are generally associated with organ transplantation but are also used to treat auto-immune diseases such as rheumatoid arthritis or multiple sclerosis[1]. Some are also used in cancer therapy[2].

Immunosuppressant drugs work by inhibiting one or more steps in the T-cell activation pathway, usually Interleukin-2 (IL-2) (sirolimus, everolimus)[3] or calcineurin (cyclosporin, tacrolimus)[4]. This inhibits the development of graft-versus-host (GVH) reactions (generally referred to as transplant rejection) but also leaves the patient more vulnerable to common illnesses and infections[5]. Monitoring the level of immunosuppressant drugs is essential to preventing GVH issues while enabling the patient to live a reasonably normal life.

One issue in immunosuppressant testing is that the absorption of the medication will depend on a number of factors such as diet and other medications[6]. Thus there may be as much as an 8-fold difference in the trough level of an immunosuppressant drug even though the patient is compliant with the dosing regimen[7].

Another issue in testing is that drugs may be combined to provide protection for the transplant but avoid undesirable side effects[8]. Any test method must be able to accurately detect and differentiate multiple drugs.

Immunoassay is most commonly used for therapeutic drug monitoring of immunosuppressive drugs as the methods are easily integrated into general laboratory workflows. However, other medications or supplements and drug metabolites may also react in an immunoassay, causing an overestimation of the drug levels[9]. Liquid chromatography – tandem mass spectroscopy (LC-MS/MS) is much more precise, but historically requires extensive preparation and technical expertise.

The Thermo Scientific™ Cascadion™ SM Immunosuppressants Panel is the first LC-MS/MS Immunosuppressant Drug assay to be available on an easy-to-use random-access analyzer. The assay measures only the bioactive drugs and displays individual results to the operator.

No off-line sample handling is required. The Cascadion SM Clinical Analyzer performs all required pretreatment processes automatically, so no specialized techniques or knowledge is required. After a brief training, any laboratory personnel can run the analyzer.

The Cascadion SM Immunosuppressants panel is the first LC-MS/MS Immunosuppressant Drugs assay to be run on an easy to use random-access clinical analyzer. The assay maximizes accuracy by measuring only the drugs ordered for the sample and by excluding inactive metabolites.

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Benefits

- No pretreatment required
- Automated sampling from primary collection tubes
- All assay materials provided ready to use by Thermo Fisher Scientific
- No interference from metabolites
- Results consistent across all laboratories using the Cascadion SM Clinical Analyzer and the Cascadion SM Immunosuppressants Panel

No pretreatment

The Internal Standard formulation includes materials which cause proteins to precipitate as well as lysing blood cells in whole-blood samples. This eliminates the need for manual pretreatment and the associated possibility of human error.

Automated sampling from primary collection tubes

Primary whole blood sample tubes can be uncapped, placed into sample racks, and the barcode scanned by the analyzer-LIS link. This saves time as well as reducing the chance for human error.

Random-access capability

Samples can be run as they are received, with no need to batch samples. Urgent samples can be easily added to the worklist and loaded into the sample compartment without pausing the analyzer.

Time to first result is approximately 30 minutes if the analyzer is idle. After that, results will be released at approximately 2.5-minute intervals. This provides physicians with timely, accurate results to assist in patient care.

Results consistent across laboratories

Results from laboratory-developed tests may vary between laboratories as the individual laboratories create their own calibrators. Because Thermo Fisher Scientific provides all reagents and consumables, results will be consistent from one laboratory to another, even if those laboratories are widely separated.

Performance Characteristics

All testing was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

General Information

	Cyclosporin A	Tacrolimus	Everolimus	Sirolimus
Analytical Measuring Range (ng/mL)	8.5 – 920	0.85 – 34.5	0.85 – 34.5	0.85 – 34.5
Sample Material	Human whole blood	Human whole blood	Human whole blood	Human whole blood
Calibration Frequency	30 days	30 days	30 days	30 days
Internal Standard on-board stability	30 days	30 days	30 days	30 days

Specificity

Assay specificity was evaluated according to CLSI EP07 and EP37. To establish any potential interference from endogenous and exogenous substances on the quantitative determination of immunosuppressive drugs, substances with similar chemical structures and other endogenous and exogenous compounds were evaluated.

The test samples were prepared by spiking pooled whole blood samples with the analytes to two medical decision concentrations with the potentially interfering compounds; compounds showing a ≤10% bias were designated non-interfering.

Compound	Test Concentration	Compound	Test Concentration
Bilirubin & Bilirubin Conjugated	0.60 mg/mL	Human anti-mouse antibodies	Normal Human Level
Biotin	3.5 µg/mL	Human Gamma-Globulin	93.5 mg/mL
Cholesterol	5 mg/mL	Rheumatoid Factor	1350 IU/mL
Creatinine	150 ug/mL	Triglycerides	15 mg/mL
Hematocrit	15% - 60%	Uric Acid	235 µg/mL
Human Albumin	100 mg/mL	Vitamin B12	1.0 ng/mL
Erythromycin	138 µg/mL	Rifampin	48 µg/mL
Fluconazole	25.5 µg/mL	Sulfasalazine	75 µg/mL
Lidocaine	15 µg/mL	Tobramycin	33 µg/mL
Prednisolone	1.2 µg/mL	Leflunomide	126 µg/mL
Prednisone	99 ng/mL	Mycophenolic acid	42 µg/mL

Assay Precision

Assay precision was evaluated according to the guidelines in CLSI EP05-A3. Three levels of test sample per analyte were created from pooled donor samples and were analyzed on each of 2 analyzers. Sample order was randomized.

				Within-run		Between-run		Between-day		Within-Laboratory	
Analyte	Test Sample	N	Mean (ng/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Cyclosporine A	Low	80	58.38	1.29	2.2	0.00	0.0	1.62	2.8	2.07	3.6
	Med	80	353.27	8.18	2.3	3.85	1.1	5.08	1.4	10.37	2.9
	High	80	714.28	11.70	1.6	0.00	0.0	11.61	1.6	16.48	2.3
Everolimus	Low	80	3.51	0.12	3.3	0.03	0.9	0.08	2.4	0.15	4.2
	Med	80	5.73	0.20	3.4	0.14	2.4	0.00	0.0	0.24	4.2
	High	80	8.85	0.30	3.4	0.09	1.0	0.00	0.0	0.31	3.5
Sirolimus	Low	80	4.04	0.17	4.2	0.09	2.3	0.04	1.1	0.20	4.9
	Med	80	7.83	0.27	3.4	0.00	0.0	0.08	1.0	0.28	3.6
	High	80	15.41	0.54	3.5	0.00	0.0	0.18	1.2	0.57	3.7
Tacrolimus	Low	80	3.57	0.10	2.9	0.00	0.0	0.08	2.1	0.13	3.6
	Med	80	9.04	0.23	2.6	0.17	1.8	0.00	0.0	0.29	3.2
	High	80	16.28	0.48	3.0	0.21	1.3	0.24	1.4	0.58	3.5

Ordering Information

Item	Part Number	Item	Part Number
Cascadion SM Immunosuppressants Panel Internal Standard	10018777	Quick Connect Cartridge H	992200
Cascadion SM Immunosuppressants Panel Calibrator Set	10018772	Solvent A	MB123-1
Cascadion SM Immunosuppressants Panel Control 1	10018773	Solvent B	MB122-1
Cascadion SM Immunosuppressants Panel Control 2	10018774	Solvent C	MB124-1
Cascadion SM Immunosuppressants Panel Control 3	10018775	Probe Wash Solution 1	T001252500
Cascadion SM Clinical Analyzer	99990000	Probe Wash Solution 2	T001262500
Cascadion SM Clinical Analyzer Accessory Cabinet	990700	Probe Wash Solution 3	MB124-212

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