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Manual

IDK® Immunosuppressants LC-MS/MS Kit

For the in vitro determination of four immunosuppressants in whole blood

Valid from 2023-03-16

REF **KM9100**

Σ 300

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

The intended use of this device is to aid in the therapeutic drug monitoring (TDM) of four immunosuppressant medications (i.e. cyclosporin A, everolimus, sirolimus & tacrolimus) by the assessment of medicine levels by determination of these levels of immunosuppressants in whole blood, performed by automated quantitative LC-MS/MS assay technology conducted by laboratory professionals.

2. INTRODUCTION

Therapeutic drug monitoring (TDM) is based on the assumption that there is a relationship between blood concentration and clinical effect (therapeutic improvement and adverse effects). It is also assumed that there is a concentration range of the drug that is characterised by maximum efficacy and maximum safety, the "therapeutic window". The Immundiagnostik LC-MS/MS assay for the measurement of four different immunosuppressants from whole blood allows the monitoring of the titer of the analytes by their quantitative determination and thus enables an individual dose adjustment of the medication.

Patients who receive a organ transplantation (SOT) or a stem cell transplantation (SCT) require immunosuppressant drugs to suppress the patients' immune response to the transplant and prevent rejection of the transplant. The goal with immunosuppression is to induce donor specific tolerance for the transplant with minimal impairing of the patients' defenses or increasing the susceptibility to infections. The most common immunosuppressants prescribed are the calcineurine inhibitors cyclosporine and tacrolimus, and the mTOR inhibitors sirolimus and everolimus. These immunosuppressants are used for the prophylaxis and treatment of graft rejection following SOT (kidney, lung, hart, and liver) and SCT [5, 6].

With cyclosporine, tacrolimus, sirolimus and everolimus, clinical variability due to inter- and intra- patient pharmacokinetic variability is seen. This pharmacokinetic variability, in combination with an excellent correlation between blood concentrations and efficacy/toxicity of the treatment and the narrow therapeutic range of the immunosuppressive drugs, makes monitoring of blood concentrations a crucial part of the treatment. The therapeutic range for each immunosuppressant drug depends on post-transplant time, concomitant immunosuppressive medication and immunological risk [7].

TDM to optimize immunosuppressive treatment and minimizing drug-related toxicity is therefore standard care in the clinical setting as well as in the outpatient setting, and the corresponding TDM is advised in the summary of product characteristics (SPC) of cyclosporine, tacrolimus, sirolimus, and everolimus [8–11]. Furthermore, a number of consensus documents on TDM of immunosuppressive drugs have been written to assist pharmacists and clinicians to individualize the treatment [3, 12].

Besides standard monitoring of drug concentrations, extra monitoring is recommended to ensure that an appropriate systemic drug exposure is maintained in case of clinical treatment failure (the occurrence of an acute rejection episode), dose adjustments or changes in the immunosuppressive regimen, a switch in (generic) formulation of the immunosuppressive drug, suspected problems with the absorption of the drug, adverse events, drug-drug interactions, relevant comorbidities and if nonadherence is suspected [3–8, 13] .

The Immundiagnostik LC-MS/MS assay for measuring immunosuppressive drugs includes cyclosporine, tacrolimus, sirolimus, and everolimus. For all these drugs, reference concentrations are based on literature and an overview of target concentrations can be found in several articles as well in the SPC of cyclosporin A, tacrolimus, sirolimus, and everolimus [3–9, 14–18].

In conclusion, immunosuppressive drugs fulfill the prerequisites for TDM, having a narrow therapeutic window, a high intra- and inter-individual pharmacokinetic variability, and an established exposure-response relationship. TDM of immunosuppressive drugs is recommended for optimal use of these drugs in clinical practice as stated in the SPC of these drugs as well as in several international guidelines and consensus documents.

3. MATERIAL SUPPLIED

Cat. No.	Identifier	Kit components	Quantity
KM9100	AUTOWASH	Autosampler wash solution; ready to use	1 x 1 000 ml
	CAL1–6	Calibrators 1–6; lyophilised (see product specification for concentration)	2 vials (à 500 µl) per level
	CTRL1–3	Controls 1–3; lyophilised (see product specification for concentration)	3 vials (à 500 µl) per level
	PREC	Precipitation solution (contains internal standard)	3 x 55 ml
	MOPHAA	Mobile phase A	1 x 500 ml
	MOPHAB	Mobile phase B	1 x 500 ml
	MIXSOL	Mixing solution	3 x 25 ml

For reorders of single components, please use the catalogue number followed by the identifier without space as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

The following accessories are required for the *IDK*® Immunosuppressants LC-MS/MS application (not included in the kit):

- Ultrapure water*
- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Centrifuge 10 000 *g* (at least)
- Vortex mixer
- Standard laboratory disposable plastic reagent tubes (e.g. 1.5 ml)
- LC-MS/MS system and LC-MS vials

*Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696/LC-MS grade), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

The following accessories for the *IDK*® Immunosuppressants LC-MS/MS application can be ordered seperately at Immundiagnostik AG:

- UPLC column (KM9100SP)
- all single components

Please ask for our single component price list. Please contact us for customized inquiries.

5. PREPARATION, STORAGE AND STABILITY OF REAGENTS

Note: Please unpack the kit components from the transport packaging immediately upon receipt and follow the instructions for storage conditions printed on the product labels. In general, all components should be stored protected from light, dry and at the specified storage temperature.

All components are for LC-MS/MS use only, components may also contain other ingredients than those listed as active ingredients below which might influence the measurement. All declared stabilities are only valid in case of no bacterial contamination.

Calibrators and controls

Handling:

Always remove the cap and rubber plug carefully (in order to avoid loss of content).

Reconstitute the calibrators and controls as follows:

- Reconstitute each calibrator and control with exactly 500 µl distilled or deionised water and incubate for 15 min at room temperature.
- Next, mix the component thoroughly to make sure that all dry material has dissolved; do not shake too vigorously to avoid foam formation.
- Handle the prepared component as a patient sample during the test procedure.

Stability and storage:

Before reconstitution:	2–8 °C	Until expiry date printed on the product label.
After reconstitution:	-20 °C	4 weeks

Precipitation solution

Handling:

The components are liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 3 weeks if closed and stored at 2–8 °C.

Mobile phases A and B

Handling:

The component is liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C or 6 weeks on the UHPLC.
Store at RT	Before first opening the component can be stored for 3 months at room temperature.

Autosampler wash solution

Handling:

The components are liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C or 2 weeks on the UHPLC.

Mixing solution

Handling:

The components are liquid and ready for use.

Stability and storage:

Store at 2–8 °C — After first opening the component can be used for 3 weeks if closed and stored at 2–8 °C.

6. STORAGE, STABILITY AND PREPARATION OF SAMPLE

Interferences & limitations

Visual evidence of lipemia, homolysis or icterus (hyperbilirubinemia) and/or older age of the specimen may affect the performance.

Storage and stability

Use whole blood (EDTA- and Heparin-tubes).

Avoid freeze-thaw cycles.

Samples can be stored: 3 months at -20 °C.

Sample preparation

	1.5 ml reaction tube	96-well plate
1.	Pipet 50 µl sample, CAL or CTRL into one reaction tube respectively well.	
2.	Add 200 µl MIXSOL.	
3.	Vortex the tube for at least 30 s.	Shake the plate for 15 min.
4.	Add 500 µl PREC.	
5.	Vortex the tube for at least 30 s.	Shake the plate for 15 min.
6.	Centrifuge for 5 min at 10 000 *g* (or more).	
7.	Transfer the supernatant (step 6.) into a suitable LC-MS vial or 96-well plate for the LC-MS autosampler.	
8.	Injection in the LC-MS/MS (see application note).	

7. LC-MS/MS METHOD

Please refer to the application note or contact lcms@immundiagnostik.com for the LC-MS/MS method parameters.

8. EXAMPLES OF CHROMATOGRAMS

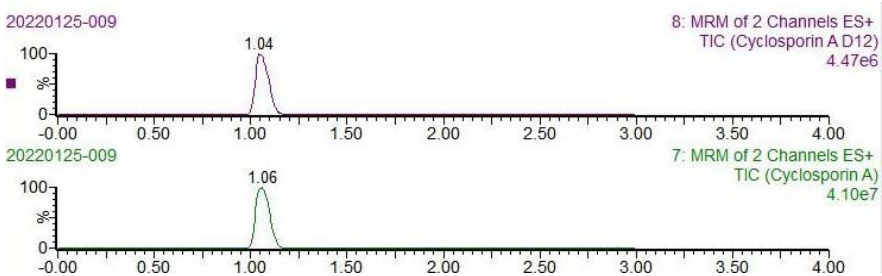

Fig. 1: Chromatogram (recorded with Waters UHPLC I-Class and the LC-MS/MS system Xevo TQS) for the internal standard (top) and analyte (bottom) of cyclosporin A.

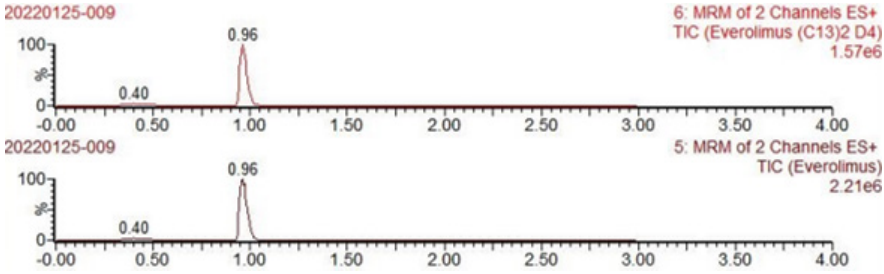

Fig. 2: Chromatogram (recorded with Waters UHPLC I-Class and the LC-MS/MS system Xevo TQS) for the internal standard (top) and analyte (bottom) of everolimus.

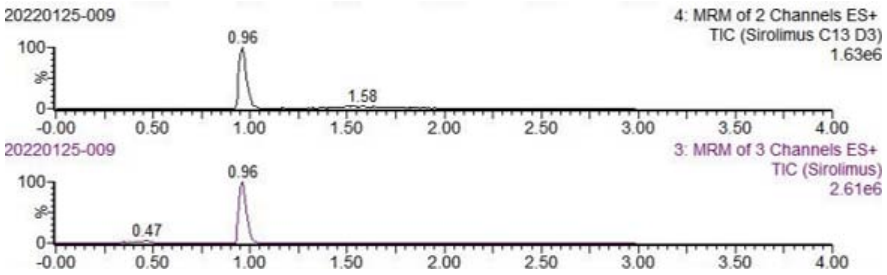

Fig. 3: Chromatogram (recorded with Waters UHPLC I-Class and the LC-MS/MS system Xevo TQS) for the internal standard (top) and analyte (bottom) of sirolimus.

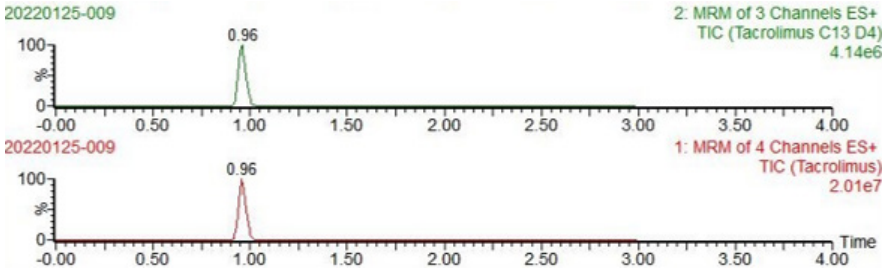

Fig. 4: Chromatogram (recorded with Waters UHPLC I-Class and the LC-MS/MS system Xevo TQS) for the internal standard (top) and analyte (bottom) of tacrolimus.

9. QUALITY CONTROL

Control samples should be analysed with each run. The results of the control samples are used to confirm the accuracy of the method. The test results may not be valid, if one or more values of the quality control sample are outside the acceptable range (see product specification).

Results from LC-MS/MS and reference values

The test gives a certain value for the measurand, which must always be compared with corresponding reference values in order to interpret it for the respective patient.

Since the substances measured do not occur naturally in the human body, no classical reference values can be used as with endogenous substances. For pharmaceutical substances, other values apply, such as normal dosage, upper limit and toxicity. As this information is (patient) specific and technical in nature, the Immundiagnostik AG refers to the healthcare professional under whose supervision the tests are performed [1–4].

Always consult a trained medical professional with expertise in the field for which this kit is designed for the interpretation of results.

Interpretation of the results of this test also depends significantly on the individual characteristics of the patient involved. Immundiagnostik AG recommends taking these inputs into consideration as well.

10. TESTCHARACTERISTICS

Repeatability - intra-day precision

	CV		
Sample	**Control 1 [%]**	**Calibrator 6 [%]**	**Patient sample [%]**
Cylosporin A	2.0	2.1	1.7
Everolimus	5.1	1.8	3.8
Sirolimus	5.5	2.2	3.2
Tacrolimus	3.8	1.3	1.9

Reproducibility - inter-day precision

	CV		
Sample	**Control 1 [%]**	**Calibrator 6 [%]**	**Patient sample [%]**
Cylosporin A	4.6	4.9	5.0
Everolimus	6.5	5.2	7.9
Sirolimus	5.3	4.3	5.5
Tacrolimus	3.6	5.9	4.4

Measuring range with limit of quantification (LOQ)

Analyte	**µg/l (mmol/l)**
Cylosporin A	< 20–2 500 (< 16.6–2 078.8)
Everolimus	< 1.4–195(< 1.5–203.5)
Sirolimus	< 1.5–190(< 1.6–207.8)
Tacrolimus	< 1.4–200(< 1.7–248.8)

11. PRECAUTIONS

- Human material used in the kit components was tested and found to be negative for HIV1/2-, HBV- and HCV-antibodies, Hepatitis B-surface antigen, HIV1- and HCV-RNA, HBV-DNA (NAT) Still, all kit components should be treated as potentially infectious.
- The GHS symbols indicated on the individual components and specifications of the material safety data sheets (available on request from Immundiagnostik AG) must be noted. When working with these reagents, the legal protective precautions must be adhered to.
- The test components contain organic solvents. Avoid contact with skin or mucous membranes.

12. DISPOSAL

Autosampler wash solution (AUTOWASH), mobile phase A (MOPHAA), mobile phase B (MOPHAB), precipitation solution (PREC) and mixing solution (MIXSOL) must be disposed as non-halogenated solvents.

Calibrators (CAL1–6) and controls (CTRL1–3) should be disposed as potentially infectious material in accordance with local regulations.

13. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- Plugs and caps of different reagents should not be swapped.
- The individual components of the kit are designed for a maximum of the specified number of test runs. Any part of the components that has already been used must not be reused, but must be disposed of properly in accordance with local regulations.

14. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- *IDK*® is a trademark of Immundiagnostik AG.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- Please contact Immundiagnostik AG if one or more components of the kit are damaged, missing (see material supplied) or precipitates are visible in the ready-to-use solutions.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG together with a written complaint.

15. REFERENCES

1. https://tdm-monografie.org/ciclosporine/
2. https://tdm-monografie.org/everolimus/
3. https://tdm-monografie.org/sirolimus/
4. https://tdm-monografie.org/tacrolimus/
5. Allison. Immunosuppressive Therapy in Transplantation. Nurs Clin North Am. 2016;51(1):107-20
6. Medication Guidelines for Solid Organ Transplants, BC Transplant Society, Canada 2021
7. Brunet et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Ther Drug Monit 2019;41(3):261-307
8. Summary of product characteristics Prograft Available via: https://www.ema.europa.eu

Used symbols:

	Temperature limitation		Catalogue number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Contains plasma derivatives or human blood		Consult instructions for use
	Consult specification data sheet		Do not re-use
	Unique Device Identification		Contains material of animal origin
	Medicinal substance		Contains material of human origin