



Immunosuppressants in whole blood

4180 P IMM

Instructions for use, LC-MS/MS assay

Diagnostix BV
De Plassen 4
9902 SE Appingedam
The Netherlands

SRN: NL-MF-000000236

UDI-DI: 08720514311790

+31 (0)596 - 20 10 62
info@diagnostix.com
techsupport@diagnostix.com

Document version: 1.3
Replaces: 1.2
Date of release: 10-04-2025

(EU) 2017/746 - IVD Medical Device

Contents

Contents	2
1. General Information.....	4
1.1 Information for the Device	4
1.2 Intended Purpose.....	4
1.2.1 Measurands.....	4
1.2.2 Function	4
1.2.3 Specific Information indented to be provided.....	4
1.2.5 Required Specimen	4
1.2.5.1 Conditions for collection, handling and preparation of specimen	5
1.2.6 Testing Population	5
1.3 Intended User.....	5
1.4 Test Principle.....	5
1.5 Clinical Background.....	5
1.6 Notice Regarding Serious Incidents.....	6
2. Components and Accessories	7
2.1 Description of Components.....	7
2.1.1 Calibrators and Controls	7
2.1.1.1 Handling	7
2.1.1.2 Stability and Storage.....	8
2.1.1.3 Metrological Traceability.....	8
2.1.2 Deproteinization Solution + Internal Standard	8
2.1.2.1 Handling	8
2.1.2.2 Stability and Storage.....	8
2.1.3 Zinc Sulphate Solution.....	9
2.1.3.1 Handling	9
2.1.3.2 Storage and Stability.....	9
2.1.4 Mobile Phases.....	9
2.1.4.1 Handling	9
2.1.4.2 Stability and storage	9
2.1.5 Autosampler Washing Solution	10
2.1.5.1 Handling	10
2.1.5.2 Stability and storage	10
2.2 List of components provided.....	10
2.3 Separately available materials and components	11
3. Warnings, precautions, measures and limitations of use	12
3.1 General.....	12
3.1.1 Potentially infectious material.....	12
3.2 Interferences & Limitations.....	12
3.3 CMR substances.....	12
3.4 Disposal.....	12

4. Assay procedure	13
4.1 Settings and procedure.....	13
4.1.1 Required instruments and LC modules	13
4.2 The analytical system.....	13
4.2.1 Preparation of the analytical system	13
4.2.2 Starting the analytical system	13
4.3 LC-MS/MS Parameters and Condition.....	14
4.3.1 LC Parameters	14
4.3.2 Autosampler Conditions	14
4.3.3 Gradient	14
4.3.4 MS Conditions (e.g. Waters Xevo TQS)	14
4.4 Sample Preparation	16
4.4.1 Reconstitution of the lyophilised Calibrators / Controls.....	16
4.4.2 Sample preparation (whole blood, calibrator or control)	16
4.4.3 Sample Preparation with pipette robot.....	16
4.5 Interpretation of results	17
4.5.1 Examples of chromatograms.....	17
4.5.2 Results from LC-MS/MS and Reference Values	18
5. Summary of Analytical Performance Characteristics	19
5.1 Repeatability (Simple Precision)	19
5.2 Reproducibility (Complex Precision)	19
5.3 Linearity	20
5.4 Limit of Blank	20
5.5 Limit of Quantification	21
5.6 Carryover.....	21
5.7 Trueness	21
6. Summary of Clinical Performance Characteristics	22
7. Change log	22

1. General Information

1.1 Information for the Device

4180 P IMM - Immunosuppressants Reagent Set
UDI-DI: 8720514311790

For information on the individual components of this set, refer to chapter 2 of these instructions for use.

1.2 Intended Purpose

This Immunosuppressants kit is intended for the determination of four (4) immunosuppressant medications in whole blood, conducted by laboratory professionals on LC-MS/MS.

1.2.1 Measurands

Cyclosporin A

Everolimus

Sirolimus
Rapamycin

Tacrolimus

1.2.2 Function

The function of this device is to aid in the monitoring of several immunosuppressant medications, refer to paragraph 1.2.1, 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.

1.2.3 Specific Information indented to be provided

Deviant measurand values can be an indication of the following physiological or pathological states and/or conditions:

Incorrect dose/use of specified immunosuppressant medication.

1.2.5 Required Specimen

Human whole blood.

1.2.5.1 Conditions for collection, handling and preparation of specimen

Use EDTA-tubes to collect the specimens. Store at 2-8 °C.^{1 2 3 4}

1.2.6 Testing Population

Patients known or suspected to be using one of the measured immunosuppressants specified under paragraph 1.2.1.

1.3 Intended User

Laboratory Professional Use.

1.4 Test Principle

In this analytical method, 4 immunosuppressants are determined from human whole blood by LC-MS/MS. Prior to the LC-MS/MS analysis, a sample clean-up is performed to remove the sample matrix and to spike the samples with the internal standard. The prepared samples are injected into the UHPLC system and, after separation by chromatography on an analytical C-18 column, the compounds are ionized by electrospray ionization (ESI) and detected by MS/MS.

1.5 Clinical Background

Patients who receive a solid 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 and without increasing the susceptibility to infections. The most common immunosuppressants prescribed are the calcineurine inhibitors cyclosporin 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 risk⁷.

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

Therapeutic Drug Monitoring (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 TDM is advised in the Summary of Product Characteristics (SPC) of cyclosporine, tacrolimus, sirolimus, and everolimus^{8,9,10,11}. 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^{3,4,5,6,7,8,13}.

The Diagnostix kit 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 cyclosporine, tacrolimus, sirolimus, and everolimus^{3,4,5,6,7,8,9,14,15,16,17,18}.

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.

1.6 Notice Regarding Serious Incidents

Following (EU) 2017/746 Annex I, Chapter III, 20.4.1 af), any serious incident that has occurred in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

⁸ Summary of product characteristics Prograf Available via: <https://www.ema.europa.eu>

⁹ Summary of product characteristics Certican Available via: <https://www.ema.europa.eu>

¹⁰ Summary of product characteristics Rapamunde Available via: <https://www.ema.europa.eu>

¹¹ Summary of product characteristics Neoral Available via: <https://www.ema.europa.eu>

¹² Shipkova et al. Therapeutic Drug Monitoring of Everolimus: A Consensus Report. *Therapeutic Drug Monitoring*. 2016;38(2):143-69

¹³ van Gelder. European Society for Organ Transplantation Advisory Committee recommendations on generic substitution of immunosuppressive drugs. *Practice Guideline Transpl Int* 2011;24(12):1135-41

¹⁴ Rodríguez-Perálvarez et al. Tacrolimus trough levels, rejection and renal impairment in liver transplantation: a systematic review and meta-analysis. *Am J Transplant*. 2012;12(10):2797-814

¹⁵ Moes et al. Sirolimus and everolimus in kidney transplantation. *Drug Discov Today*. 2015;20(10):1243-9

¹⁶ Kahan et al. Therapeutic drug monitoring of sirolimus: correlations with efficacy and toxicity. *Clin Transplant* 2000;14(2):97-109

¹⁷ Diciolla et al. C2 and C0 values for monitoring cyclosporine therapy in stable heart transplant recipients. *Transplantation Proceedings* 2005;37:1355-1359

¹⁸ Andrews et al. Cyclosporin: revisions in monitoring guidelines and review of current analytical methods. *Ann Clin Biochem*. 2002;39:424-35

2. Components and Accessories

2.1 Description of Components

All components are for LC-MS/MS use only, components also may 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.

2.1.1 Calibrators and Controls

4181a CAL P IMM | Immunosuppressants Calibrator Set

UDI: 8720514312858

A six-point lyophilized whole blood calibrator at clinically relevant levels, refer to the value data sheet provided with each set for specific values per production batch.

4182a CON P IMM | Immunosuppressants Control Set

UDI: 8720514312865

4193a P IMM | Immunosuppressants Control I

UDI: 8720514312933

4194a P IMM | Immunosuppressants Control II

UDI: 8720514312940

4195a P IMM | Immunosuppressants Control III

UDI: 8720514312957

Three levels of lyophilized whole blood controls at clinically relevant levels for quality control purposes, refer to the value data sheet provided with each set for specific values per production batch.

2.1.1.1 Handling

Reconstitute the calibrators and controls as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Immunosuppressants Calibrator Set and Controls with exactly 250 µl distilled or deionised water using a volumetric pipette.
Note: Diagnostix strongly recommends the use of type 1 ultrapure (LC-MS grade) water.
3. Re-place the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Swirl the vial carefully, do not shake. Avoid foaming.
7. Use the preparation as a patient sample.

2.1.1.2 Stability and Storage

The stability of the calibrators and controls are:

After reconstitution:	2 - 8 °C	Tacrolimus	7 days
		Cyclosporin A, Sirolimus, Everolimus	15 days
	-20 °C	Tacrolimus	35 days
		Cyclosporin A, Sirolimus, Everolimus	90 days

The declared stated stabilities are only valid in case of no bacterial contamination

2.1.1.3 Metrological Traceability

Metrological traceability is established by comparing each batch to the highest available order of reference material, as well as the last batch produced before the current batch.

For Immunosuppressants the highest available order of reference material has been established to be LGC Standards which is a division of LGC Group. This group is the designated National Measurement Institute for chemical and bioanalytical measurements in the United Kingdom.

Refer to the Value Data Sheet for this specific set for more information.

2.1.2 Deproteinization Solution + Internal Standard

4189 P IMM | Immunosuppressants Deproteinization Solution + Internal Standard

UDI: 8720514311189

An isotopic version of the measurand, dissolved in a deproteinization solution. Used to identify and correct potential deviating values, due to errors or varying circumstances in sample preparation or within the LC-MS/MS. The deproteinization solution furthermore deproteinizes the sample to prevent pollution in the LC-MS/MS.

Active ingredient(s): [2H4]-Cyclosporin A,
[13C2,2H4]-Everolimus,
[13C,2H3]-Sirolimus
[13C,2H4]-Tacrolimus.

Methanol 75% - <100%

2.1.2.1 Handling

The Reagent is liquid and ready for use.

2.1.2.2 Stability and Storage

The stability of the internal standard is:

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C.

The declared stated stabilities are only valid in case of no bacterial contamination

2.1.3 Zinc Sulphate Solution

4190 P IMM | Immunosuppressants Zinc Sulphate Solution

UDI: 8720514311196

A solution provided to perform an extra pre-deproteinization step.

Active ingredient(s): <1% Zinc Sulphate

2.1.3.1 Handling

The Reagent is liquid and ready for use.

2.1.3.2 Storage and Stability

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C.

The declared stated stabilities are only valid in case of no bacterial contamination

2.1.4 Mobile Phases

4191 P IMM | Immunosuppressants Mobile Phase I

UDI: 8720514311202

4092 P IMM | Immunosuppressants Mobile Phase II

UDI: 8720514311219

Two mobile phases are provided to carry the sample through the LC-MS/MS. Different ratios of the mobile phases will allow different components to elute from the column at differing speeds.

Active ingredient(s):

Mobile Phase I:	2.5% -<10% Methanol <1% Formic acid
Mobile Phase II:	75% - <100% Methanol <1% Formic acid

2.1.4.1 Handling

The Reagents are liquid and ready for use.

2.1.4.2 Stability and storage

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

The declared stated stabilities are only valid in case of no bacterial contamination

2.1.5 Autosampler Washing Solution

4196 P IMM | Immunosuppressants Autosampler washing solution

UDI: 8720514311806

A solution used to clean the UHPLC-MS/MS system after use, specifically designed to remove residue from testing the measurand.

Active ingredient(s): 75% - <100% Methanol

2.1.5.1 Handling

The Reagent is liquid and ready for use.

2.1.5.2 Stability and storage

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

The declared stated stabilities are only valid in case of no bacterial contamination

2.2 List of components provided

4180 KIT P IMM - Complete Kit for immunosuppressants in whole blood

Contents (for 300 assays):

Immunosuppressants Calibrator Set (Calibrator 1 – 6)	4181a CAL P IMM	6 x 2 x 250 µl
Immunosuppressants Deproteinization Solution + Internal Standard	4189 P IMM	3 x 55 ml
Immunosuppressants Zinc Sulphate Solution	4190 P IMM	3 x 25 ml
Immunosuppressants Mobile Phase I	4191 P IMM	1 x 500 ml
Immunosuppressants Mobile Phase II	4192 P IMM	1 x 500 ml
Immunosuppressants Autosampler Washing Solution	4196 P IMM	1 x 1000 ml
Immunosuppressants Manual		

2.3 Separately available materials and components

Immunosuppressants Calibrator Set (Calibrator 1 – 6)	4181a CAL P IMM	6 x 2 x 250 µl
Immunosuppressants Deproteinization Solution + Internal Standard	4189 P IMM	1 x 55 ml
Immunosuppressants Zinc Sulphate Solution	4190 P IMM	1 x 25 ml
Immunosuppressants Mobile Phase I	4191 P IMM	1 x 500 ml
Immunosuppressants Mobile Phase II	4192 P IMM	1 x 500 ml
Immunosuppressants Autosampler Washing Solution	4196 P IMM	1 x 1000 ml

Analytical column Waters XBridge BEH C18 2,5µm 2,1x75mm XP	186006030	1 pc
Immunosuppressants Control I	4193a.10 P IMM	10 x 250 µl
Immunosuppressants Control II	4194a.10 P IMM	10 x 250 µl
Immunosuppressants Control III	4195a.10 P IMM	10 x 250 µl
Immunosuppressants Control Set	4182a CON P IMM	3 x 3 x 250 µl

3. Warnings, precautions, measures and limitations of use

3.1 General

The device and its components must only be used in line with the intended purpose by the intended user as stated in chapter 1. Due to their nature, most reagents of this device contain or are largely composed of hazardous substances. Please refer to the Safety Data Sheets (SDS) for each of the components for specific hazards and measures to be taken.

Used components should be discarded and are not suitable for re-use.

3.1.1 Potentially infectious material

The human whole blood used for manufacturing calibrators and controls was tested for the following infectious markers and found negative: HIV1/2-, HBV- and HCV-antibodies, Hepatitis B-surface antigen, HIV1- and HCV-RNA, HBV-DNA (NAT). Nevertheless, the whole blood controls should be considered as potentially infectious and treated with appropriate care.

3.2 Interferences & Limitations

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

3.3 CMR substances

No CMR substances are used in any significant quantity in the manufacturing of this kit or its components.

3.4 Disposal

For the safe disposal of the components of this kit, please refer to the safety data sheet of the component in question.

4. Assay procedure

4.1 Settings and procedure

4.1.1 Required instruments and LC modules

Using this test kit requires a UHPLC system with tandem mass spectrometer (LC-MS/MS) with the following modules:

- Autosampler
- UHPLC gradient pump
- Column heater
- Degasser

4.2 The analytical system

4.2.1 Preparation of the analytical system

- Flush the LC system excluding the column.
- Set the UHPLC pump at a flow rate of 1 ml/min and flush the system for 10 minutes with Mobile Phase I and II (50 : 50).
- Connect the column with the column heater.
(see arrow marking on the column)

After flushing the system, the equilibration is performed as follows:

- Set the UHPLC pump to a flow rate of 0.5 ml/min.
- Set the column heater to 30°C.
- Equilibrate the column for 15 minutes with Mobile Phase I.
- Start the program for the gradient and equilibrate for another 10 minutes.

4.2.2 Starting the analytical system

- Equilibrate the system.
- Check the temperature of the column.
- Initialize the injector.
- Start the programme on the LC-MS/MS system.

4.3 LC-MS/MS Parameters and Condition

Please note that the provided LC-MS/MS Parameters and Conditions are derived from the system used by Diagnostix to perform the validation of the analytical performance of this assay kit. Conditions may vary between LC-MS/MS, even between systems of the same type from the same manufacturer. End-user systems used to perform this assay may require optimization.

4.3.1 LC Parameters

UHPLC pump	Flow rate 0.5 ml/min
Mobile Phases I and II	Close the bottles to avoid alteration of RT's through evaporation of the mobile phases
Column	The column is installed in the column heater 40°C For the complete UHPLC system the backpressure should not exceed 800 bar. 1 bar = 14.5 PSI

4.3.2 Autosampler Conditions

Injection volume:	25 µL
Sample temperature:	10 °C
Runtime:	4 min
Column temperature:	40 °C ± 2 °C alarm
Needle wash:	post injection wash 12 sec.
Seal Wash:	5 minutes
Wash Solvent:	Autosampler Washing Solution; MeOH

4.3.3 Gradient

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.5	50	50	Initial
0.2	0.5	50	50	6
0.25	0.5	0	100	6
0.5	0.5	0	100	6
2	0.5	0	100	6
2.01	0.5	50	50	6
4	0.5	50	50	6

Please note that the gradient is dependent on the analyser used. End users will need to define the optimal gradient for the analyser in use.

4.3.4 MS Conditions (e.g. Waters Xevo TQS)

MS System:	Waters Xevo TQS
Ion mode:	Electrospray
Capillary voltage:	2.2 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	350°C
Desolvation gas flow:	1000L/hr
Detection mode:	ESI
Dwell time:	0.013 sec
Collision gas:	Argon

Substance	Precursor	Product
Tacrolimus	821.45	768.35
Tacrolimus	821.45	786.4

Substance	Precursor	Product
Tacrolimus C13 D4	826.5	773.5
Tacrolimus C13 D4	826.5	786.4

Substance	Precursor	Product
Sirolimus	931.5	864.4
Sirolimus	931.3	882.35

Substance	Precursor	Product
Sirolimus C13 D3	935.5	864.4
Sirolimus C13 D3	935.5	882.65

Substance	Precursor	Product
Everolimus	975.6	908.45
Everolimus	975.6	926.5

Substance	Precursor	Product
Everolimus (C13)2 D4	981.6	914.5
Everolimus (C13)2 D4	981.6	932.6

Substance	Precursor	Product
Cyclosporin A	1202.8	224
Cyclosporin A	1202.8	425.1

Substance	Precursor	Product
Cyclosporin A D4	1206.9	216
Cyclosporin A D4	1206.9	428.3

These conditions are an indication, optimal values can differ slightly between different LC-MS/MS systems.

4.4 Sample Preparation

4.4.1 Reconstitution of the lyophilised Calibrators / Controls.

Refer to paragraph 2.1.1.1 and the product value data sheets.

4.4.2 Sample preparation (whole blood, calibrator or control)

1. Pipette 50 µl sample (Calibrator, Control, Patient sample) into a vial.
2. While mixing on a vortex mixer add 200 µl Zinc Sulphate Solution.
3. Whilst continuing to mix the plate, add 500 µl Internal Standard in Deproteinization Solution.
4. Centrifuge (5 min, 10000 x g or more).
5. Pipette the centrifuged supernatant into a vial or 96 well plate, which is suitable for the auto sampler in use and Inject 25 µl in the LC-MS/MS.

4.4.3 Sample Preparation with pipette robot

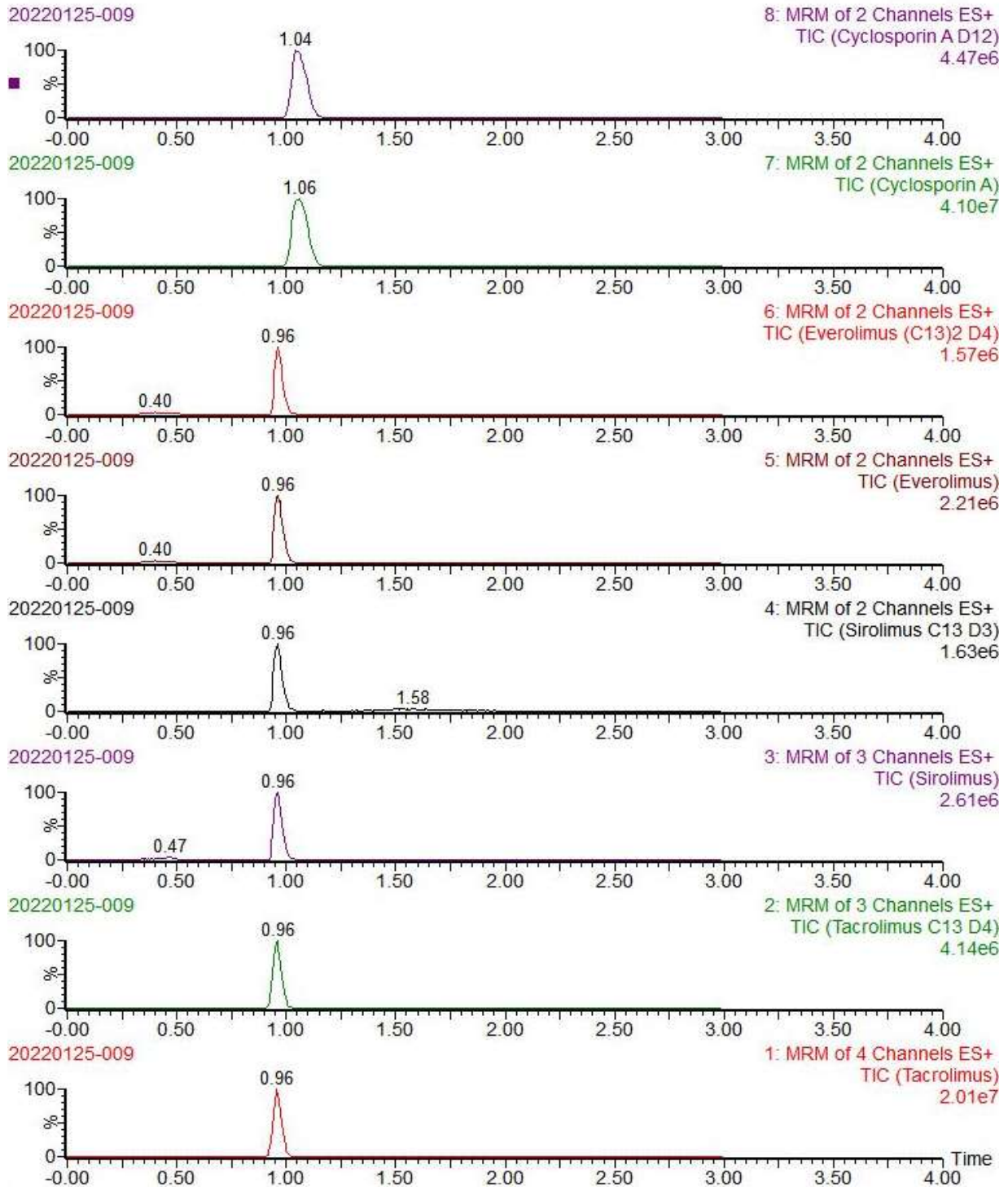
Into a 2 ml 96 well plate:

1. Add 50 µl sample (Calibrator, Control, Patient sample) into a 96 well plate.
2. Whilst mixing the plate, add 200 µl Zinc Sulphate Solution.
3. Whilst continuing to mix the plate, add 500 µl Internal Standard in Deproteinization Solution.
4. Once mixing is complete, centrifuge (5 min , 10000x g or more).
5. Transfer the samples Into a 1 ml 96 well collection plate for injection on the UHPLC/MS/MS system (Needle placement 2 mm) or inject directly off the pellet (Needle placement 10 mm) 25 µl in the LC-MS/MS.

4.5 Interpretation of results

4.5.1 Examples of chromatograms

Example chromatogram of a Patient sample, recorded with a Waters LC-MS/MS TQS:



4.5.2 Results from LC-MS/MS and Reference Values

The assay will result in a certain value for the measurand, which will need to be compared to applicable reference values to be interpreted for the specific patient.

For illustrative purposes only, an example of reference values for this device can be used as follows:

As the measured substances do not naturally occur in the human body scientific literature states the reference values as not applicable. For pharmaceutical substances different levels apply such as regular dosage, upper limit and toxicity. As this information is so (patient) specific and of a technical nature Diagnostix refers to the medical expert under whose authority testing is conducted^{1,2,3,4}.

The inclusion of this information is required by Annex I, section 20.4.1 (v) of the IVDR. Diagnostix employs no medically trained professionals and can only indicate possible ways of interpreting results based on published scientific literature. Always consult a trained medical professional with expertise in the area of interest for this kit for interpretation of results.

Interpretation of the results of this test also depends in a significant way on the individual characteristics of the patient involved. Diagnostix recommends taking these inputs into consideration as well.

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. Summary of Analytical Performance Characteristics

Analytical performance characteristics have been defined by validation of the assay according to IVDR parameters, using EP Evaluator to calculate statistical data from the acquired raw data.

5.1 Repeatability (Simple Precision)

The Repeatability, or Simple Precision, was analyzed by measuring a patient sample, Immunosuppressants control I and Immunosuppressants calibrator 6 twenty times from one sample within a two hour period. From these results the Coefficient of Variation (CV) is calculated and compared to the precision verification goal: for low samples 10% and for normal and high samples 7.5%.

	CV		
Sample	4193 P IMM Control I Lot: 14H21/07	4188 P IMM Calibrator 6 Lot: 14H21/06	Patient
Cyclosporin 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 %

5.2 Reproducibility (Complex Precision)

The Reproducibility, or Complex Precision, was analyzed by measuring a patient sample, Immunosuppressants control I and Immunosuppressants calibrator 6 in duplicate twenty times. Each time the sample preparation had a variance (different analyst, pipet, day, reagent temperature and/or calibration). This to simulate twenty different days in a laboratory. From these results the Coefficient of Variation (CV) is calculated and compared to the precision verification goal which for low samples is 15% and for normal and high samples 12.5%.

	CV		
Component	4193 P IMM Control I Lot: 14H21/07	4188 P IMM Calibrator 6 Lot: 14H21/06	Patient
Cyclosporin 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 %

5.3 Linearity

The linearity was analyzed by preparing a series of incrementally increasing Immunosuppressants concentrations in whole blood two times. These samples were measured in two fold from which the linearity was verified (<10% deviation from line fit and CV <15%) and upper limit of detection was determined.

Analyte	Linearity (µg/l)
Cyclosporin A	2350
Everolimus	60
Sirolimus	130
Tacrolimus	120

5.4 Limit of Blank

The LOB is calculated by comparing twenty zero responses with five known (non-zero) responses. From the zero responses the standard deviation (SD) was calculated and multiplied by two. The response found by this calculation was multiplied by a factor calculated from the known non-zero responses to be able to calculate the LOB concentration.

Please note that this concentration depends highly on which analyzer is used. Diagnostix advises users to verify this concentration for every analyzer used.

Analyte	LOB (µg/l)
Cyclosporin A	0.379
Everolimus	0
Sirolimus	0.817
Tacrolimus	0.0838

5.5 Limit of Quantification

The Limit of Quantification (LOQ) was analyzed by preparing a series of seven incrementally decreasing Immunosuppressants concentrations in whole blood. These samples were prepared and measured twelve times under varying conditions (different analyst, pipet, day, reagent temperature and/or calibration). This to simulate different conditions in a laboratory. From the acquired data the LOQ was determined by calculating the concentration at the CV cutoff value of 20% (a confidence interval of 95% is taken into account). When this concentration deviates more than 20% from the target value the sample with lowest concentration that does meet both these criteria is set as the LOQ.

Please note that this concentration depends highly on which analyzer is used. Diagnostix advises users to verify this concentration for every analyzer used. When the verified LOQ exceeds the concentration of calibrator 2 please increase your injection volume or exclude calibrator 2 from the calibration.

Analyte	LOQ (µg/l)
Cyclosporin A	15
Everolimus	0.28
Sirolimus	0.34
Tacrolimus	1.3

5.6 Carryover

To verify that there is no carryover two samples were prepared; one low (calibrator 2) and one high (calibrator 6). The samples were divided into eleven low samples and ten high samples. The samples were then measured in a particular order after which the datasets were analyzed. The mean and standard deviation of two concentrations is measured, High-Low and Low-Low (these are the low level concentrations measured after another low level measurement). The two mean values are subtracted to get the carryover value. This value may not exceed the error limit which is set to 3*SD (low-low concentrations).

None of the components showed signs of carryover.

5.7 Trueness

The trueness* of the method was determined by measuring different calibrators and controls from third party commercial manufacturers, Recipe and Chromsystems. Currently no adequate higher order reference material is available to Diagnostix. The results are compared to the target values which for low samples may not exceed 20% and for normal and high samples may not exceed 15%.

All components met the requirements.

* trueness instead of accuracy due to the lack of certified reference material.

6. Summary of Clinical Performance Characteristics

Not available at the time of this version of the instructions for use.

7. Change log

Version Change	Changed section	What has been changed	Date
From V1.2 to V1.3	2.3 2.1.1.2	Removed analytical column 4180-C. Updated in-use stability calibrators and controls	10-APR-2025
From V1.1 to V1.2	2.1.2 4.3.4	The internal standard for Cyclosporin-A has been changed from a D12 to a D4 isotope. This also means that the Precursor and Fragment M/Z values have changed. This change does not affect patient outcomes.	18-DEC-2024

8. 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 Prograf Available via: <https://www.ema.europa.eu>
- [9] Summary of product characteristics Certican Available via: <https://www.ema.europa.eu>
- [10] Summary of product characteristics Rapamunde Available via: <https://www.ema.europa.eu>
- [11] Summary of product characteristics Neoral Available via: <https://www.ema.europa.eu>
- [12] Shipkova et al. Therapeutic Drug Monitoring of Everolimus: A Consensus Report. Therapeutic Drug Monitoring. 2016;38(2):143-69
- [13] van Gelder. European Society for Organ Transplantation Advisory Committee recommendations on generic substitution of immunosuppressive drugs. Practice Guideline Transpl Int 2011;24(12):1135-41
- [14] Rodríguez-Perálvarez et al. Tacrolimus trough levels, rejection and renal impairment in liver transplantation: a systematic review and meta-analysis. Am J Transplant. 2012;12(10):2797-814
- [15] Moes et al. Sirolimus and everolimus in kidney transplantation. Drug Discov Today. 2015;20(10):1243-9

[16] Kahan et al. Therapeutic drug monitoring of sirolimus: correlations with efficacy and toxicity. Clin Transplant 2000;14(2):97-109

[17] Diciolla et al. C2 and C0 values for monitoring cyclosporine therapy in stable heart transplant recipients. Transplantation Proceedings 2005;37:1355-1359

[18] Andrews et al. Cyclosporin: revisions in monitoring guidelines and review of current analytical methods. Ann Clin Biochem. 2002;39:424-35