ARCHImedline®

IVD CE

Instruction Manual

Methylmalonic acid in Serum / Plasma

Important!
Please read the full instructions for use before starting the assay procedure.

UDI	N/a
	MMA E Version 2, ARCHImedline GmbH
REF	A100; A101
	ARCHImedline GmbH, Leberstraße 20/2, 1110 Vienna, Europe

Table of contents

1 Name and purpose

This Methylmalonic acid in Serum / Plasma kit is an in vitro diagnostic medical device and is intended for the quantitative determination of methylmalonic acid from human serum / plasma by HPLC coupled with tandem mass spectrometry (LC-MS/MS).

The kit is intended for use by professional users in clinical and medical laboratories only.

2 Clinical background and test principle

2.1 Clinical background

Vitamin B12 (cobalamin) is an essential nutrient and plays an important role for the normal functioning of the human organism. The coenzyme form of vitamin B12 (coenzyme B12) participates in two metabolic key positions. One of these reactions is the vitamin B12-dependent conversion of methylmalonyl-coenzyme A (CoA) to succinyl-CoA [1]. In cases of Vitamin B12 deficiency, methylmalonyl-CoA accumulates and methylmalonic acid (MMA) is subsequently released (see Figure 1) [1, 2].

Accordingly, vitamin B12 deficiency results in quantitative accumulation of MMA in blood and urine. This occurs already in the early stages of insufficiency, i.e. when vitamin B12 levels still appear “normal” (see below), making MMA a sensitive, early biomarker for intracellular, functional vitamin B12 deficiency [2].

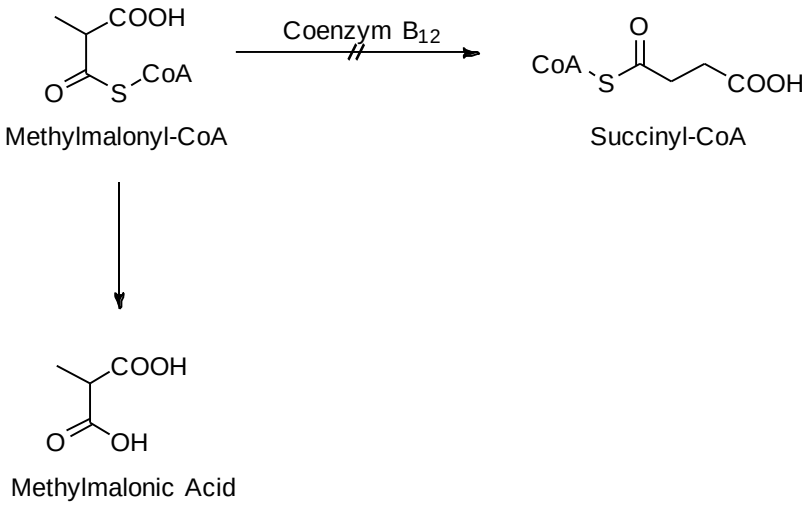


Figure 1 Vitamin B12 deficiency and release of MMA

In contrast, the determination of vitamin B12 in serum (as total vitamin B12), which is frequently used due to its cost efficiency, does not show adequate selectivity and sensitivity at the lower reference level range (below 400 pmol/l) [1]. As such this can lead to potential false negative diagnosis in cases of intracellular functional vitamin B12 deficiency, where vitamin B12 levels appear normal (> 156 pmol/l). In such

cases however the serum MMA is already significantly increased (> 300 nmol/l) and clearly indicates the deficiency [1].

In these particular cases holotranscobalamin (Holo TC) and homocysteine* will be determined in addition to total vitamin B12 and MMA. Holo TC is the intracellularly utilised form of vitamin B12 and, as a precursor of coenzyme B12, is required for the conversion of MMA and homocysteine. A metabolically manifested vitamin B12 deficiency will thus be indicated by lowered levels of Holo TC and by increased levels of MMA and homocysteine [1 - 3].

The determination of MMA can be performed from serum, plasma, and urine. Serum samples are generally used for MMA determination, as this matrix is used for parallel cobalamin level tests. The advantage of determination from serum therefore is the sample availability. Furthermore, nutrition seems to have less influence on the MMA serum level than is the case with urine [4, 5]. Additional measurement of creatinine is also necessary for the determination from urine, as the MMA/creatinine ratio is required for data interpretation [5].

The advantage of determination from urine however lies in the significantly higher MMA levels, which facilitate the analyses. In cases of patients with impaired renal function serum MMA measurements may provide false positive results due to reduced urinary MMA excretion [6]. However calculation of the urine MMA/creatinine ratio can compensate for this [7].

Mass spectrometry based methods have been widely tested for the determination of MMA. GC/MS has been routinely applied to the quantitation of MMA, however, due to the requirement of derivatization prior to analysis an alternative method with less time-consuming sample preparation and hence faster turn-around time is of continued interest. The application of LC-MS/MS methods to MMA determination has received increased attention in the last few years, which however still bears some challenges due to the low endogenous concentration of MMA, the highly polar nature, low molecular weight, low pKa and dicarboxylic acid structure. Furthermore chromatographic separation from the naturally occurring structural isomer succinic acid (SA), present in physiological concentrations approximately 50 times higher than MMA, is critical and not elementary. Many methods hence require lengthy sample preparation steps such as solid-phase extraction, derivatization, evaporation and/or ultrafiltration, and can also show sub-optimal resolution from succinic acid [8, 9].

This method was developed for the routine analysis of methylmalonic acid (MMA) in human serum and plasma samples. Sample preparation is simple and rapid, and analogous for the different biological matrices. Calibration is performed using lyophilized serum calibrators at clinically relevant levels. Lyophilized serum controls are also available for quality assurance. An isotope-labelled internal standard (d3-methylmalonic acid) is used in order to compensate for matrix effects and measurement variations. Samples are analyzed using negative ion electrospray in MRM mode for maximum sensitivity and selectivity.

2.2 Test principle

In this analytical method MMA is determined from human serum or plasma by HPLC coupled with electrospray-tandem mass spectrometry (LC-MS/MS). The routine analysis of MMA is primarily performed from serum. However, the methodology presented here can also be applied to plasma (K2EDTA) (see also section 6).

Prior to the LC-MS/MS analysis a short manual sample clean-up is performed in order to remove the sample matrix and to spike with the internal standard (sample preparation, see section 7.2 and 7.3).

The prepared samples are injected into the LC-MS/MS system for chromatographic separation of the compounds. The analytes are then ionized using electrospray ionization (ESI). Electrospray ionization is a soft ionization technique where a strong electric field is applied to the liquid passing through the ESI-capillary of the MS-source. The ions are mostly preformed in solution before desorption and then transferred into the ion path of the tandem mass spectrometer which consists of three quadrupoles (two mass selectors connected by a collision cell).

Measurement of the analytes is carried out in MRM mode (MRM: Multiple Reaction Monitoring). In this mode only selected ions (known as “precursor ions”) with a defined mass/charge (m/z) ratio are isolated in the first quadrupole and subsequently transferred into the collision cell, where they are fragmented by impact with an inert gas (argon or nitrogen) at defined voltage settings. Among the fragments generated (known as “product ions”) only those with a defined m/z ratio can pass the third quadrupole for final detection. In this way the MRM mode ensures a selective identification and quantification of the target analytes.

The Optimization Mix is provided for the optimization of the MS/MS parameters and for the test run of the analytical system (see section 7.5). The calibration of the analytical system is performed by use of Serum Calibrators. For this purpose, a 4-Level Serum Calibrator Set is provided (see section 7.5.3).

Quality control is performed by use of Serum Controls. These controls are available in two different concentrations (see section 7.5.4).

The kit components have to be used in accordance with this user manual. The kit is not designed for combination with components from other manufacturers.

3 Safety and performance precautions

3.1 Performance precautions

This test procedure is intended for professional use only and requires experience in the field of clinical analysis. To avoid cross-contamination, following good laboratory practices is a prerequisite. Use only calibrated devices and disposable tips. Prevent cross-contamination by not opening used reaction vessels or plates and discarding them immediately in designated waste containers.

Do not use the materials past the expiration date. Store reagents according to directions as improper storage may affect stability. Do not mix solutions or materials from different lots. Record the lot number used for each test to ensure sample traceability.

3.2 Safety precautions

Kit components such as mobile phases and reagents are chemical preparations and may therefore contain hazardous substances. Safety-relevant information on the respective product can be found in the corresponding safety data sheets.

The serum calibrators and controls were prepared from human material and tested for the absence of various known pathogens. Irrespective of this, the products must be classified as potentially infectious. For this reason, we recommend that you take the same precautions when handling these products as when handling patient samples (see Attachment I and safety data sheets for the product).

Wipe up spilled reagents with absorbent material. Wear laboratory protective equipment (laboratory goggles, lab coat and nitrile gloves). The passage time of the protective gloves must be checked by each individual laboratory.

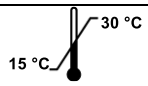
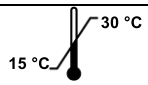
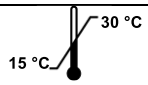
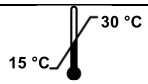
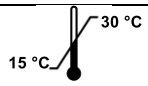
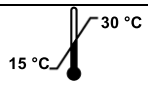
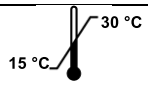
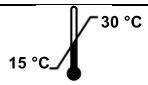
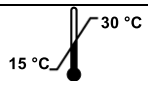
4 Storage and stability

Please unpack the kit components from the transport packaging immediately upon receipt and follow the instructions for storage conditions indicated on the product labels and Table 1. Unused components, stored under appropriate conditions can be used until the expiry date indicated on the product label.

Reagents and mobile phase bottles must be closed tightly and stored immediately under the required conditions after use. Provided proper use and storage procedures are followed, the lifetime of opened reagents is indicated on the respective product data sheet. Mobile phases can be used for one month after initial opening given proper storage conditions.

For storage conditions and lifetime of the Optimization Mix as well as the calibrators and controls (lyophilized / after reconstitution) please also refer to the respective product data sheets.

Table 1 Storage conditions & Kit components

Product.-Nr.		Product description	Storage condition	
REF	A100-001	Mobile Phase A for Methylmalonic acid		Store at 15 – 30°C
REF	A100-002	Mobile Phase B for Methylmalonic acid		Store at 15 – 30°C
REF	A100-003	Mobile Phase B		Store at 15 – 30°C
REF	A100-005	Washing Solution for Methylmalonic acid		Store at 15 – 30°C
REF	A100-006	Optimization Mix for Methylmalonic acid		Store at -25 – -18°C
REF	A100-010	Precipitant solvent with Internal Standard for Methylmalonic acid		Store at -25 – -18°C In-use at 2-8°C 21days
REF	A100-011	Serum Calibrator Set, lyophil. (Level 0 – 3) for Methylmalonic acid		Store at 2 – 8°C*
REF	A100-012	Serum Controls, lyophil. (Level I-II) for Methylmalonic acid		Store at 2 – 8°C*
REF	A100-046	Deepwell-Plates 1.2mL		Store at 15 – 30°C
REF	A100-049	1,5mL Sample Preparation Vials		Store at 15 – 30°C
REF	A100-050	Protective Foil		Store at 15 – 30°C
REF	A100-090	Analytical Column for Methylmalonic acid		Store at 15 – 30°C
REF	A100-092	Pre-column for Methylmalonic acid		Store at 15 – 30°C

*Refers to the lyophilized product. For storage conditions after reconstitution, please refer to the product data sheet

5 Equipment and materials

5.1 Provided materials

Order number	Description	Amount
A100-A	**Methylmalonic acid kit for Deepwell plates**	1
	Content:	
	Mobile Phase A for Methylmalonic acid	2 x A100-001
	Mobile Phase B for Methylmalonic acid	1 x A100-002
	Washing solution for Methylmalonic acid	1 x A100-005
	Optimization Mix for Methylmalonic acid	1 x A100-006
	Precipitation solvent with Internal Standard for Methylmalonic acid	4 x A100-010
	Serum Calibrator-Set, lyophil. (Level 0-3) for Methylmalonic acid	1 x A100-011
	Serum Control-Set, lyophil., Level I, II for Methylmalonic acid	1 x A100-012
	Deepwell-Plates 1.2mL	4 x A100-046
	Protective Foil	1 x A100-050
	Analytical Column for Methylmalonic acid	1 x A100-090
	Pre Column for Methylmalonic acid	1 x A100-092
A100-B	**Methylmalonic acid refill-kit for Deepwell plates**	1
	Content:	
	Mobile Phase A for Methylmalonic acid	2 x A100-001
	Mobile Phase B for Methylmalonic acid	1 x A100-002
	Washing solution for Methylmalonic acid	1 x A100-005
	Precipitation solvent with Internal Standard for Methylmalonic acid	4 x A100-010
	Serum Calibrator-Set, lyophil. (Level 0-3) for Methylmalonic acid	1 x A100-011
	Serum Control-Set, lyophil., Level I, II for Methylmalonic acid	1 x A100-012
	Deepwell-Plates 1.2mL	4 x A100-046
	Protective Foil	1 x A100-50
A101-A	**Methylmalonic acid kit for sample preparation in 1.5 mL reaction tubes**	1
	Content:	
	Mobile Phase A for Methylmalonic acid	1 x A100-001
	Mobile Phase B for Methylmalonic acid	1 x A100-003
	Washing solution for Methylmalonic acid	1 x A100-005
	Optimization Mix for Methylmalonic acid	1 x A100-006
	Precipitation solvent with Internal Standard for Methylmalonic acid	2 x A100-010

	Serum Calibrator-Set, lyophil. (Level 0-3) for Methylmalonic acid	1 x A100-011
	Serum Control-Set, lyophil., Level I, II for Methylmalonic acid	1 x A100-012
	1.5mL Sample Preparation Vials	3 x A100-049
	Analytical Column for Methylmalonic acid	1 x A100-090
	Pre Column for Methylmalonic acid	1 x A100-092

A101-B	**Methylmalonic acid refill-kit for sample preparation in 1.5 mL reaction tubes**	1
	Content:	
	Mobile Phase A for Methylmalonic acid	1 x A100-001
	Mobile Phase B for Methylmalonic acid	1 x A100-003
	Washing solution for Methylmalonic acid	1 x A100-005
	Precipitation solvent with Internal Standard for Methylmalonic acid	2 x A100-010
	Serum Calibrator-Set, lyophil. (Level 0-3) for Methylmalonic acid	1 x A100-011
	Serum Control-Set, lyophil., Level I, II for Methylmalonic acid	1 x A100-012
	1.5mL Sample Preparation Vials	3 x A100-049

	Components sold separately	
A100-001	Mobile Phase A for Methylmalonic acid	2,4L
A100-002	Mobile Phase B for Methylmalonic acid	2,2L
A100-003	Mobile Phase B for Methylmalonic acid	1L
A100-005	Washing solution for Methylmalonic acid	1L
A100-006	Optimization Mix for Methylmalonic acid	1mL
A100-010	Precipitation solvent with Internal Standard for Methylmalonic acid	150mL
A100-011	Serum Calibrator-Set, lyophil. (Level 0-3) for Methylmalonic acid	4 x 1 x 2 mL
A100-012	Serum Control-Set, lyophil., Level I, II for Methylmalonic acid	2 x 1 x 2 mL
A100-046	Deepwell-Plates 1.2mL	4pcs.
A100-049	1.5mL Sample Preparation Vials	250pcs.
A100-050	Protective Foil	16pcs.
A100-090	Analytical Column for Methylmalonic acid	1pcs.
A100-092	Pre Column for Methylmalonic acid	1pcs.

5.2 Required materials

Required laboratory equipment

Laboratory equipment	Manufacturer and article number
Pipettes	Eppendorf or equivalent
Benchtop Centrifuge suitable for Deepwell plates	Eppendorf 5804 with Plate rotor or equivalent
Benchtop Centrifuge suitable for 1,5mL-Reaction tubes	Eppendorf or equivalent
Vortex	VWR or equivalent
Orbital shaker	Heidolph Titramax 100 or equivalent

Required consumables

Consumables	Manufacturer and article number
1,5mL HPLC Vials with screwcap	Carl Roth LC03.1 and LC11.1
Pipette tips	Eppendorf or equivalent

Required special equipment

Special equipment	Manufacturer and article number
HPLC	Binary pumping system capable of performing up until 400bar at a flow rate of 1.2mL/min Autosampler capable of injecting from VT54 vials or Deepwell plates (kit dependent); Injection volume 1-20µL Column oven capable of supplying 40°C

	Vendor e.g. Shimadzu Nexera
Mass spectrometer	Triple quadrupole instrument with high enough sensitivity for a mass range of 50-1000 m/z; calibrated negative ion mode Vendor e.g. Shimadzu 8050

6 Specimens and preparatory treatment

The routine analysis of MMA is primarily performed from serum. If serum is not available, plasma (K2EDTA) can also be used.

The samples can be stored at least 3 days at room temperature (15 - 30 °C), at least 7 days at temperatures between 2 - 8 °C and at least 3 months at temperatures below -18 °C (multiple freeze-thaw cycles should be avoided).

7 Assay performance

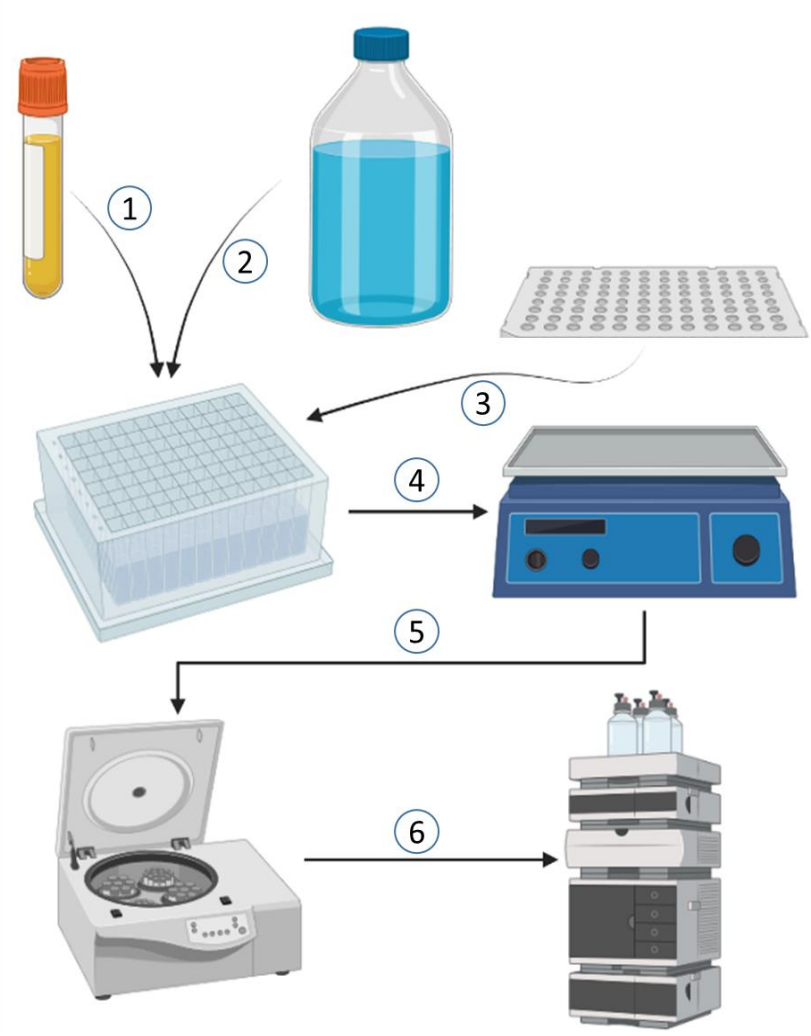
7.1 Reconstitution of the lyophilized serum calibrators / controls

Serum Calibrators and Serum Controls (see section 5.1) are lyophilized and must be reconstituted before use. Information regarding reconstitution, analyte concentrations, storage and stability is indicated in the respective product data sheets.

7.2 Workflow Deepwell plates

7.2.1 Flow diagram

1. 100µL Sample/Calibrator/QC
2. 400µL Precipitant (chilled)
3. Cover with foil
4. Shake for 3 minutes at 1050 rpm
5. Centrifuge for 10 minutes at RT (3700 rpm)
6. Transfer plate to 5°C Autosampler



7.2.2 Sample distribution

Transfer 100µL of Calibrator/Control/Sample in an empty deep well.

7.2.3 Precipitation

Before using the precipitation solution, it must be stored at 2-8°C in a fridge for at least 1h. Add 400µL of chilled precipitant (contains internal standard) to each calibrator as well as control/sample and seal the plate tightly with the foil. The plate is then put on an orbital shaker for 3 minutes at 1050 rpm. Then the plate is centrifuged at 3700rpm

for 10 minutes at RT (medium acceleration and deceleration). After usage of precipitation solution, it can be stored at 2-8°C in a fridge for up to 21 days.

7.2.4 LC-MS/MS Analysis

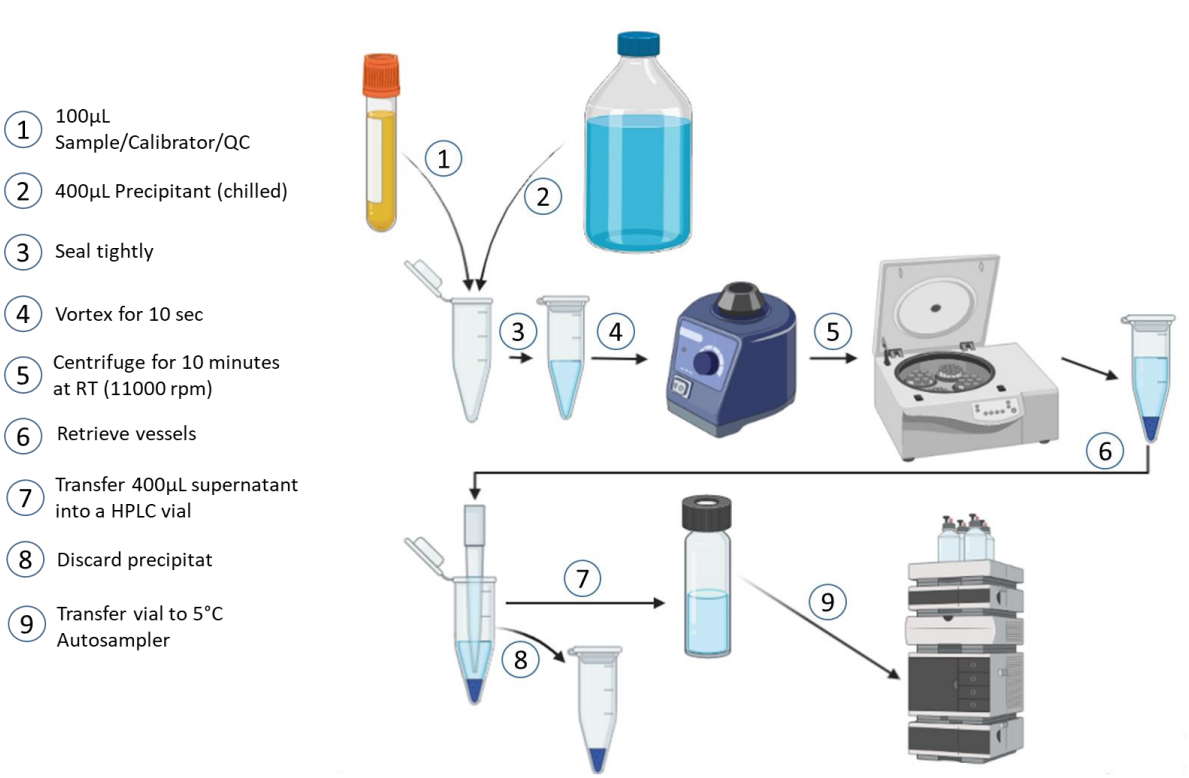
Inject 5µL sample in LC-MS/MS system. The injected sample volume can be adjusted according to the instrument's sensitivity needs.

7.2.5 Sample stability

Prepared samples are stable for 24h at 5°C.

7.3 Workflow 1.5mL reaction tubes

7.3.1 Flow diagram



7.3.2 Sample distribution

Transfer 100µL of Calibrator/Control/Sample in an empty 1.5mL reaction tube.

7.3.3 Precipitation

Add 400µL of chilled precipitant (contains internal standard) to each calibrator as well as control/sample and seal tightly. Each reaction tube is then vortexed for 10 seconds until a clumped precipitate is visible. The tubes are then centrifuged at 11000rpm for 10 minutes at RT (medium acceleration and deceleration). 400µL of supernatant is then transferred to an empty 1.5mL HPLC vial, capped and put on Autosampler.

7.3.4 LC-MS/MS Analysis

Inject 5µL sample in LC-MS/MS system. The injected sample volume can be adjusted according to the instrument's sensitivity needs.

7.3.5 Sample stability

Prepared samples are stable for 24h at 5°C.

7.4 Setup of the analytical instrument

7.4.1 Flushing of the LC-System

Connect the LC modules except for the column. Place the outlet capillary in a waste container. Specify a flow rate of 1 ml/min for the HPLC pump and flush the LC system with 10 ml of Mobile Phase A/B (Mobile Phase A/B = 50:50). Then connect the analytical column in the column oven. When connecting the analytical column, make sure that the direction of flow is correct (note the arrow on the column!).

Please also ensure that suitable fittings are available for column. A new fitting should be used for the connection and adapted to the column. For questions about the professional connection, please contact ARCHIMED Life.

7.4.2 Equilibration of the LC-System

After rinsing (see section 7.4.1), equilibration is carried out as follows:

Specify a flow rate of 0.2 ml/min for the HPLC pump and set a temperature of 40 °C on the column oven. Equilibrate the column for 10 min with mobile phase starting conditions (5% mobile phase B).

Then stop the pump and connect the outlet capillary of the analytical column to the tandem mass spectrometer.

7.4.3 Instrument startup

The parameters to be set for the LC system (see Section 7.4.4) and the tandem mass spectrometer (see Section 7.4.5) are specified in the following sections. For the optimization, equilibration, and test run, as well as for the calibration of the LC-MS/MS system, please refer to Section 7.5.

For the correct operation of your tandem mass spectrometer, please refer to the corresponding operating instructions of the device manufacturer. If necessary, training by the manufacturer is required.

7.4.4 LC-Parameter

Table 2 LC-Parameters

HPLC-Pump (Mobile Phase A, B):	Flowrate: 1.2 mL/min Gradient program for binary pump: see table 3 Make sure the storage bottles are tightly capped. Due to the otherwise occurring evaporation of certain components of the mobile phases, the retention times could change.
Analytical column	The analytical column is installed in the column oven (40 °C). With a flow rate of 1.2 mL/min, the initial back pressure of the analytical column should not exceed 150 bar.
Autosampler:	Injection volume 5 µL Injection interval: 3.2 min Needle wash: The injection needle should be rinsed after sample aspiration (to minimize sample carryover). To do this, use the needle rinsing settings recommended by the autosampler manufacturer. The washing solution (Order No. A100-005) should be used for rinsing.

The following gradient program is to be specified for the binary HPLC pumps:

Table 3 Binary gradient program

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]	Flow rate [mL/min]
0.00	95	5	1.2
0.20	95	5	1.2
1.20	50	50	1.2
1.21	0	100	1.2
2.00	0	100	1.2
2.01	95	5	1.2
3.00	95	5	1.2

*Please note that the gradient may need to be adjusted depending on the dead volume of the respective HPLC system.

7.4.5 MS/MS Parameter

The MS/MS parameters given in the tables below are to be regarded as guide values. In particular, the specified parameters for the mass transitions are to be understood as starting points for optimization. Since the optima can vary between the different MS/MS systems, they must be determined for the system in question (see Section 7.5.1).

The stated parameters referred to in tables 4 & 5 have been determined on a Shimadzu 8050 system.

Table 4 MS/MS-Parameter, Shimadzu 8050

	Shimadzu 8050
Ionsource	ESI
Polarity	Negative
Nebulizing Gas Flow	3 L/min
Heating Gas Flow	10 L/min
Interface Temperature	400 °C
DL Temperature	150 °C
Heat Block Temperature	400 °C
Drying Gas Flow	10 L/min

Table 5 Mass transitions, Shimadzu 8050

Substance	Precursor [m/z]	Product [m/z]	Dwell time [ms]	Collision energy
Methylmalonic acid (Quantifier)	117.1	72.95	75	11
Methylmalonic acid (Qualifier)	117.1	55.05	75	23
Methylmalonic acid - Internal Standard (Quantifier)	120.3	76.15	75	12
Methylmalonic acid - Internal Standard (Qualifier)	120.3	58.15	75	24

7.4.6 Standby mode

During breaks in operation, the HPLC pump should be switched off, the mobile phase can remain in the LC system.

The vacuum pumps of the tandem mass spectrometer (MS/MS system) should be in constant operation. To protect the ionization source and the multiplier, the MS/MS system should be switched to standby mode.

If the operation is interrupted for more than 2 days, the analytical column should be removed and stored tightly closed. If the analytical system is not used for several days, we recommend flushing the instrument with 100% mobile phase B or storing it in an aqueous-alcoholic (>30%) solvent to prevent the growth of microorganisms and to prevent valve circuits from getting stuck.

7.5 LC-MS/MS Analysis

Independent from the analytical method, the mass accuracy of the tandem mass spectrometer (MS/MS) should be checked at regular intervals. A mass calibration may be required.
For information regarding the check-up of the MS/MS system, please refer to the documentation provided by the instrument manufacturer.

7.5.1 Optimization of the tandem-mass spectrometer

The optimization of the MS/MS system includes the optimization of the ion source parameters and the substance-specific mass transitions. An optimization mix is available for this purpose. The optimization mix contains the analyte and the internal standard. If necessary, the optimization mix should be diluted with MPB according to the sensitivity of the existing MS/MS system.

7.5.2 Instrument equilibration and test run

Ensure that all lines are flushed and equilibrate the entire analysis system for at least 10 min before injecting samples.

Three injections of Mobile Phase should be performed at the beginning of each analytical series.

To perform a test run, inject the optimization mix multiple times until two consecutive chromatograms with comparable retention times and peak areas are obtained. The optimization mix must first be diluted with mobile phase according to the sensitivity of the existing MS/MS system.

7.5.3 Calibration

The calibrator set is available for calibration:

A100-011 Serum Calibrator Set lyophil. (Level 0-3)

Perform the calibration in ascending order. After reconstitution, the serum calibrators are to be prepared in the same way as patient samples (see section 7.2 or 7.3).

Freshly prepared calibrators should be used for each analytical series.

7.5.4 Controls

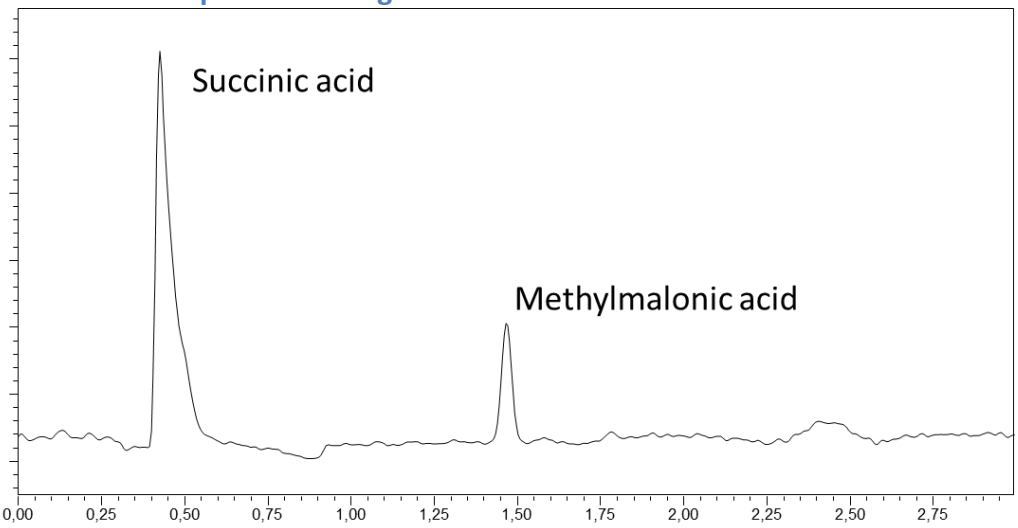
Controls in various concentrations are available for quality control of the analytical measurements:

A100-012 Serum Control Set lyophil. Level I, II

After reconstitution, the control sera should be prepared in the same way as patient samples (see Section 7.2 or 7.3).

Freshly prepared controls should be used for each analytical series. In the case of large analytical series, we recommend additional controls at the end of the series.

7.5.1 Example chromatogram



Chromatogram serum control, Level 2

7.6 Evaluation

The analytes are detected by measuring substance-specific mass transitions, see Section 7.4.5.

The analyte concentration is calculated using the internal standard method via peak areas. The calibration line is obtained from the calibrators by plotting the ratio of the "analyte/internal standard" peak area versus the "nominal" concentration. The concentrations of the analytes in the samples and the controls are calculated from the calibration curves.

In order to ensure a correct evaluation, please observe the instructions for use of the MS/MS software from the device manufacturer.

To convert [µg/L] into molar concentration [nmol/L] and vice versa, multiply by the factor from Table 6.

Table 6 Conversion factors

Analyte	Molecular weight [g/mol]	Conversion: nmol/L→ µg/L	Conversion: µg/L→nmol/L
MMA	118,09	0,118	8,468

7.7 Reference values

Table 7 Normal range for MMA

	Plasma, Serum [10]
Normal range	73–271 nmol/l

The specified reference ranges are taken from carefully selected and current scientific literature. They are up to date as of the printing date of this document. Please note that these ranges do not reflect recommendations by the manufacturer of this device but can be used as a guide for the clinical laboratory's assessment of the reference range.

7.8 Troubleshooting

Problem	Possible cause	Corrective Measurement
Gradient profile cannot be generated	Defective HPLC pump	Check the pumps
	Air within the system	Degas the mobile phases and flush and purge the HPLC system thoroughly
	Fluctuation of the flow rate	Check the pumps
Interference signals	Injection system contaminated	Inject 10 x Mobile phase B Check flush port solvent level Clean/exchange needle seat assembly and/or injection valve
	Sample vials contaminated	Use new vials
	Vial septum contaminated	Use another septum
	Mobile phase contaminated	Change the mobile phases and flush the system
	Column(s) (guard / analytical column) contaminated	Change the guard / analytical column
	Mass resolution too low	Optimize mass resolution
	System not correctly configured	Check all connections
Decrease of sensitivity	Mass resolution too high/low	Optimize the mass resolution
	Shift of mass calibration	Recalibrate MS/MS system
	Leakage of injection valve	Check the injector
	Mass spectrometer contaminated	Clean the mass spectrometer
	Ion source contaminated	Clean the ion source
No signals	Defective HPLC pump	Check the pumps
	MS/MS system not ready for operation	Check the MS/MS system
	Injector defect	Check injector
No gas supply	Gas bottle is empty	Replace the gas bottle
	Defective compressor	Check the compressor
	Defective nitrogen generator	Check the nitrogen generator
	Inlet gas pressures are not	Regulate the inlet gas

	within the specified range	pressures
High fluctuations of signals	Gas flow rate instable	Check the gas lines
	Fluctuation of the flow rate	Check the HPLC pumps
	Spray instable	Check the spray needle capillary and clean or exchange, if necessary
No vacuum	Defective vacuum pumps	Check the pre- and high-vacuum pumps
	Leakage within the vacuum system	Check the vacuum tubes and fittings

8 Performance characteristics

The following results were obtained with a Shimadzu LCMS-8050 MS/MS system

8.1 Linearity, LOD, LLOQ

Table 8 MMA Ranges for linearity and Quantification limits

	Serum/Plasma	
	[µg/L]	**[nmol/L]**
Linearity	18.75 - 1500	159 - 12712
LOD	4.68	39.71
LLOQ	15.61	132.38

8.2 Recovery

For MMA mean recovery rates between 90-100% were obtained

8.3 Precision

Samples with three different concentrations were used to determine the intra- and inter-assay accuracy of the method. The analyte concentrations are included in Table 9 along with the results.

Table 9 Results Intra - & Interday precision

	Concentration [µg/L]	**Concentration [nmol/L]**	**Intraday Precision CV [%]; n=10**	**Interday Precision CV [%]; n= 80**
Level 1	31.9	270	6	10
Level 2	69.5	588	3	6
Level 3	125	1060	1	12

9 Quality control

The control sera (see 5.1) must be carried along with each run. It is recommended to take part in a round robin test every year.

10 Limitations

The Methylmalonic Acid Kit only works with the specified sample matrices. Furthermore, only concentrations within the calibration range can be determined.

11 Disposal

Used consumables and solvents should be disposed of in specially designated waste containers according to national guidelines.

12 Contact information

You can always contact us with any question. Therefore, please use the following contact information:

E-Mail: info@archimedline.com

Phone: +43 664 1283579

Web: www.archimedlife.com

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Leberstraße 20/2, 1110 Vienna, Austria

13 Symbols

According to the EU Directive 98/79/EG on in-vitro diagnostics (IVD), the following IVD symbols are used on the product labels and in this manual:

	Manufacturer information
	Expiration date
	Batch designation
	Reference number
	Temperature range
	Instruction manual
	For *in-vitro* diagnostic purpose
	Unique device identifier
	Single use only
	Contains biological material of human origin

13.1 Hazard symbols

	Danger Highly flammable liquid and vapor. Keep away from heat/sparks/open flames/hot surfaces - No smoking. Use explosion-proof electrical/ventilation/lighting/equipment. Wear protective gloves/protective clothing/eye protection/face protection. Take anti-static measures.
	Danger Harmful if swallowed, in contact with skin or if inhaled. Causes serious eye irritation. Wear protective gloves/protective clothing/eye protection/face protection.

14 Disclaimer

N/A

15 Version

V2

16 Literature

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[8] Carvalho, V.M., Kok, F., Determination of serum methylmalonic acid by alkylative extraction and liquid chromatography coupled to tandem mass spectrometry, (2008) Analytical Biochemistry, 381, 67-73.

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[11] Norman, E.J., Urinary Methylmalonic Acid Test May Have Greater Value than the Total Homocysteine Assay for Screening Elderly Individuals for Cobalamin Deficiency, Clinical Chemistry 2004, 50 (8), 1482-1483

17 Attachment I - Dangers and Safety Instructions

Attention must be paid to following information on hazardous substances and appropriate safety measures must be taken. Further information can be found in our safety data sheets.

Pictograms	Dangers and Safety Instructions
Mobile phase A - Ref. A100-001	
	H225 Flammable liquid H302 Acute toxicity (oral) H312 Acute toxicity (dermal) H332 Acute toxicity (inhalation) H319 Eye irritation P210 Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Do not smoke P233 Keep container tightly closed P261 Avoid breathing mist/vapor P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/... P305+P951+P338 In case of eye contact: Rinse cautiously with water for several minutes. If possible, remove contact lenses. Continue rinsing. P312 Call a poison center/doctor if you feel unwell P370+P378 In case of fire: Use sand, carbon dioxide or chemical powder to extinguish. P403 + P235 Keep in a well-ventilated place. Store at cool temperature P501 Dispose of contents/container to industrial incinerator
Mobile phase B - Ref. A100-002 & -003	
	H225 Flammable liquid H302 Acute toxicity (oral) H312 Acute toxicity (dermal) H332 Acute toxicity (inhalation) H319 Eye irritation P210 Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Do not smoke P233 Keep container tightly closed P261 Avoid breathing mist/vapor P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/... P305+P951+P338 In case of eye contact: Rinse cautiously with water for several minutes. If possible, remove contact lenses. Continue rinsing. P312 Call a poison center/doctor if you feel unwell

	P370+P378 In case of fire: Use sand, carbon dioxide or chemical powder to extinguish. P403 + P235 Keep in a well-ventilated place. Store at cool temperature P501 Dispose of contents/container to industrial incinerator
Optimization Mix - Ref. A100-006	
	H225 Flammable liquid H302 Acute toxicity (oral) H312 Acute toxicity (dermal) H332 Acute toxicity (inhalation) H319 Eye irritation P210 Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Do not smoke P233 Keep container tightly closed P261 Avoid breathing mist/vapor P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/... P305+P951+P338 In case of eye contact: Rinse cautiously with water for several minutes. If possible, remove contact lenses. Continue rinsing. P312 Call a poison center/doctor if you feel unwell P370+P378 In case of fire: Use sand, carbon dioxide or chemical powder to extinguish. P403 + P235 Keep in a well-ventilated place. Store at cool temperature P501 Dispose of contents/container to industrial incinerator
Precipitation solution with internal standard - Ref. A100-010	
	H225 Flammable liquid H302 Acute toxicity (oral) H312 Acute toxicity (dermal) H332 Acute toxicity (inhalation) H319 Eye irritation P210 Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Do not smoke P233 Keep container tightly closed P261 Avoid breathing mist/vapor P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/... P305+P951+P338 In case of eye contact: Rinse cautiously with water for several minutes. If possible, remove contact lenses. Continue rinsing. P312 Call a poison center/doctor if you feel unwell P370+P378 In case of fire: Use sand, carbon dioxide or chemical powder to extinguish. P403 + P235 Keep in a well-ventilated place. Store at cool temperature

	P501 Dispose of contents/container to industrial incinerator
Autosampler washing solution - Ref. A100-005	
	H319 Eye irritation P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/... P305+P951+P338 In case of eye contact: Rinse cautiously with water for several minutes. If possible, remove contact lenses. Continue rinsing.