09230815500V1.0

MTX

ONLINE TDM Methotrexate

CÔNG TY TRÁCH NHIỆM HỮU HẠN ROCHE VIỆT NAM
Trương Thị Tố Hoa
Trưởng phòng Đăng ký, Đối ngoại và Quản lý Chất lượng

cobas®

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
09230815190	09230815500	ONLINE TDM Methotrexate (100 tests)	System-ID 216 1 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

09533699190	MTX Calibrator (2 x 5 mL)	Code 20456	
04521536190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 20310 Code 20311 Code 20312	

English

System information

MTX: ACN 21610

Intended use

In vitro test for the quantitative determination of methotrexate in human serum and plasma on **cobas c** systems. The determination of methotrexate is used for monitoring levels of methotrexate to ensure appropriate therapy.

Summary

Methotrexate (MTX) is an antifolate that inhibits the enzyme dihydrofolate reductase, thereby inhibiting DNA synthesis.[1,2,3] It is used for treatment of a number of neoplastic conditions such as acute lymphoblastic leukemias and osteosarcoma. MTX is also used in the treatment of severe, uncontrolled psoriasis, polyarticular juvenile idiopathic arthritis and rheumatoid arthritis.[3,4] Particularly in cancers, it is administered at high dose (HDMTX), while low doses are used in autoimmune diseases.[5] MTX toxicity is generally correlated to dose and exposure time and it is only tolerable when a rescue agent is administered. As such, leucovorin (5-formyltetrahydrofolate, folinate) and its main circulating metabolite, 5-methyltetrahydrofolate, provide an alternative source of intracellular tetrahydrofolates entering the folate cycle downstream of dihydrofolate reductase, and thus, help to avoid life-threatening toxicities due to high-dose methotrexate.[1] Leucovorin is usually administered in patients receiving intermediate and high MTX doses. The administration of rescue agents is guided by daily measurements of serum/plasma methotrexate. The leucovorin dose must be adjusted in proportion to the MTX concentration.[1,2,3,6]

Furthermore, the decision if glucarpidase should be administered to the patient is supported by methotrexate concentrations. Glucarpidase is a rescue agent, which rapidly cleaves MTX into inactive metabolites (DAMPA; 4-deoxy-4-amino-N10-methylpteroic acid).[1,3,7,8] These metabolites interfere with methotrexate immunoassays resulting in overestimation of the serum/plasma methotrexate concentration. Therefore, a method that is able to separate methotrexate from DAMPA should be used after glucarpidase administration.[1,8]

For instructions for MTX monitoring and rescue agent administration refer to methotrexate, leucovorin/folinate and glucarpidase prescribing information and standard guidelines.

Test principle

The ONLINE TDM MTX assay is a homogeneous enzyme-immunoassay. It is a two-reagent system used for the detection of methotrexate in serum and plasma. In this technology drug hapten attached to the enzyme glucose 6 phosphate dehydrogenase (G6PDH) serves as the binding partner to anti-methotrexate antibody. A competitive reaction to a limited amount of specific anti-methotrexate antibody takes place between the enzyme bound hapten and free methotrexate in the sample. Enzyme activity is reduced with bound antibody. Only active enzymes reduce NAD^+ to NADH. The rate of NADH formation during the reaction correlates to the methotrexate concentration and is measured photometrically.

Reagents - working solutions

R1 Anti-methotrexate antibody (rabbit monoclonal); NAD, G6P, bovine serum albumin in water; preservative

R3 Methotrexate hapten conjugated to G6PDH; bovine serum albumin in buffer; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sampling tubes.
Plasma: Li-Heparin, Na-Heparin, K_2- and K_3-EDTA plasma

MTX

ONLINE TDM Methotrexate

Stability: 24 hours capped at 15-25 °C

14 days capped at 2-8 °C

26 weeks capped at -20 °C (± 5 °C)

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Do not induce foaming of specimens. Specimens can be repeatedly frozen and thawed up to 3 times.

Invert thawed specimens several times prior to testing.

Falsely elevated serum/plasma concentrations may be detected due to contamination, in case the specimen is drawn from the same lumen of the central venous catheter during or shortly after the end of the HDMTX infusion.[1]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Select Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

Test definition

Reporting time	10 min		
Wavelength (sub/main)	415 /340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R3	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.0 µL	–	–
Decreased	7.0 µL	2.0	91.0
Increased	2.0 µL	–	–

Calibration

Calibrator	S1-6: MTX Calibrator
Calibration mode	non-linear
Calibration frequency	Full calibration • after reagent lot change • every 2 weeks • as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: The MTX Calibrator is traceable to USP material and LC-MS/MS.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample in the unit µmol/L (µg/mL).

Conversion factor: µmol/L x 0.45444 = µg/mL

Limitations - interference

Specimens from patients who received glucarpidase as a high dose methotrexate rescue therapy should not be tested with the MTX assay. These samples contain significant concentrations of DAMPA, a cleavage product that is generated from MTX by glucarpidase. DAMPA crossreacts significantly with the assay and methotrextate results would be falsely elevated. Oncologists on the clinical team should notify the laboratory when glucarpidase is administered.

See the "Specific performance data" section of this document for information on substances tested with this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

Criterion: Recovery within ± 0.0200 µmol/L of initial value at MTX concentrations of approximately 0.1 µmol/L and recovery within ± 10.0 % of initial value at MTX concentrations of approximately 1 µmol/L.

Icterus:[9] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[9] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[9] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1000 IU/mL.

Total protein: No significant interference from total protein in the concentration range of 2-12 g/dL.

As with any assay employing rabbit antibodies, the possibility exists for interference by human anti rabbit antibodies (HARA) in the sample, which could cause falsely lowered result.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[10]

There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator’s manual.

Limits and ranges

Measuring range

0.0400-1.20 µmol/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:14 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 14.

Samples with MTX concentrations above the extended measuring range with rerun function can be diluted with water. The recommended dilution is

1:50 (automatically by the analyzers) or 1:200 manually. The concentration of the undiluted sample must be > 16 µmol/L. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Lower limits of measurement

Limit of Blank	= 0.0250 µmol/L
Limit of Detection	= 0.0350 µmol/L
Limit of Quantitation	= 0.0400 *µmol/L*

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation from n ≥ 60 measurements of low concentration samples over several independent series. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation for methotrexate is 0.0400 µmol/L determined in accordance with the guidelines in CLSI document EP17-A2, based on a minimum of 48 determinations; and a total error goal of 30 % calculated using the RMS error model.

Expected values

Methotrexate serum concentrations are highly variable depending on dosage and route of administration in different indications, pharmacokinetics such as hepatic and renal function, coadministration of other drugs and other clinical factors.[3,4] To decrease the risk of severe methotrexate toxicity, the rescue agent leucovorin is administered in intermediate- and high-dose methotrexate therapy. Administration is guided by methotrexate concentrations[3,4] and the leucovorin dose must be increased with increased MTX concentrations.[1] According to the cited prescribing information of Leucovorin calcium injection,[6] the following MTX thresholds are the basis for leucovorin dosing:

Normal methotrexate elimination in HDMTX[6]

Hours after MTX administration	MTX concentration
24	approximately 10 µmol/L
48	approximately 1 µmol/L
72	approximately 0.2 µmol/L

Delayed late methotrexate elimination in HDMTX[6]

Hours after MTX administration	MTX concentration
72	above 0.2 µmol/L
96	above 0.05 µmol/L

Delayed early methotrexate elimination in HDMTX[6]

Hours after MTX administration	MTX concentration
24	above 50 µmol/L
48	above 5 µmol/L

In high dose MTX therapy, plasma concentrations above 1000 µmol/L can be reached, for instance in osteosarcoma patients.[1] Methotrexate monitoring and leucovorin administration should be continued until methotrexate plasma target concentrations are reached (typically, below 0.1 or 0.2 µmol/L).[1,6]

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* *µmol/L*	*SD* *µmol/L*	*CV* *%*
Control 1	0.0802	0.00361	4.5
Control 2	0.494	0.00360	0.7
Control 3	0.856	0.00514	0.6
Human Serum 1	0.0627	0.00330	5.3
Human Serum 2	0.104	0.00281	2.7
Human Serum 3	0.198	0.00290	1.5
Human Serum 4	0.590	0.00430	0.7
Human Serum 5	1.06	0.00607	0.6

Intermediate precision	*Mean* *µmol/L*	*SD* *µmol/L*	*CV* *%*
Control 1	0.0848	0.00587	6.9
Control 2	0.494	0.00514	1.0
Control 3	0.856	0.00575	0.7
Human Serum 1	0.0668	0.00675	10.1
Human Serum 2	0.104	0.00568	5.5
Human Serum 3	0.202	0.00592	2.9
Human Serum 4	0.590	0.00525	0.9
Human Serum 5	1.07	0.00842	0.8

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

Methotrexate values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using LC-MS (x).

Sample size (n) = 105

Deming Weighted

y = 1.032x - 0.000831 µmol/L

r = 0.997

The sample concentrations were between 0.0429 and 1.19 µmol/L.

Methotrexate values for human serum samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 62

Passing/Bablok[11]

y = 0.993x + 0.00290 µmol/L

r = 1.000

The sample concentrations were between 0.0472 and 1.19 µmol/L.

Analytical specificity

DAMPA 4-[(2,4-Diaminopteridin-6-yl) methyl-methylamino] benzoic acid was tested for cross-reactivity on the **cobas c** 503 analyzer and can cross-react between 50 % and 150 %.

The assay interference was evaluated with the main methotrexate metabolite 7-Hydroxymethotrexate on the **cobas c** 503 analyzer using 200 times more 7-Hydroxymethotrexate versus MTX concentration[12] and for

the following substances and metabolites on the **cobas c** 503 analyzer. Criterion: Recovery within ± 0.0200 µmol/L of initial value at MTX concentrations below 0.2 µmol/L and recovery within ± 10.0 % of initial value at MTX concentrations above 0.2 µmol/L. No interference was found.

Drug	Drug conc. tested (mg/L)
Folic acid	0.44
Folinic acid (Leucovorin)	1420
Dihydrofolic acid	443
5-Methyl-tetrahydrofolic acid	459
Tetrahydrofolic acid	445

The assay interference was evaluated for the following common drugs on the **cobas c** 503 analyzer. No interference was found.

Drug	Drug conc. tested (mg/L)
(±)-6-Methyl-5,6,7,8-tetrahydropterin-dihydrochloride	254
5-Fluorouracil	90
6-Mercaptopurin	1.48
Acetaminophen	156
N-Acetylcysteine	150
Acetylsalicylic acid	30
Adriamycin	580
Ampicillin-Na	75
Ascorbic acid	52.5
Carbamazepine	45
Cefoxitin	750
Chloramphenicol	78
Cisplatin	15
Cyclophosphamide	549
Cyclosporine	1.8
Cytosine	111
Digoxin	0.039
Disopyramide	16.8
Doxycyclin	18
Erythromycin	138
Furosemide	15.9
Gabapentin	26.7
Heparin	3300 IU/L
Hydrochlorothiazide	1.13
Ibuprofen	219
Isoproterenol hydrochloride	0.0595
Levodopa	7.5
Lidocaine	15
Methyldopa	22.5
Metronidazole	123
Naproxen	360
Phenobarbital	690
Phenylbutazone	321
Phenytoin	60.0
Prednisolone	1.2
Prednisone	0.099
Pyrimethamine	249
Rifampicin	48
Sulfamethoxazole	405
Theophylline	60
Triamteren	0.585
Trimethoprim	42
Vancomycin	120
Vinblastine	811
Vincristine	825

References

1 Ramsey LB, Balis FM, O' Brien MM, et al. Consensus Guideline for Use of Glucarpidase in Patients with High-Dose Methotrexate induced Acute Kidney Injury and Delayed Methotrexate Clearance. Oncologist 2018;23(1):p 52-61.

2 Milone MC and Shaw LM. "Therapeutic Drugs and Their Management" in Tietz Textbook of Laboratory Medicine, 7th edition, ISBN: 978-0-323-77572-4, Copyright (c) 2023 by Elsevier, Inc, 2023.

3 Hospira, Inc. METHOTREXATE injection, solution Package insert [revised 2022 Aug; cited 2023 Mar 10]. Available from: "https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=0d63ba29-b692-41b4-87e8-351265c8273f"

4 medac GmbH; Methotrexate 100 mg/ml solution for injection - Prescribing information [updated 07-Jun-2023; cited 15-Sep-2023]. Available from: "https://www.medicines.org.uk/emc/product/8504/smpc"

5 Silva MF, Ribeiro C, Goncalves VMF, et al. Liquid chromatographic methods for the therapeutic drug monitoring of methotrexate as clinical decision support for personalized medicine: A brief review. Biomed Chromatogr 2018;32(5): p. e4159.

6 Fresenius Kabi USA, LLC. LEUCOVORIN CALCIUM injection Package insert [revised 2020 Jan; cited 2023 Mar 10]. Available from: "https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=4b72d1fd-f389-45d9-b43b-510b45c92263"

7 European Medicine Agency. Voraxaze (glucarpidase) - An overview of Voraxaze and why it is authorised in the EU, 2022. Available from: "https://www.ema.europa.eu/en/documents/overview/voraxaze-epar-medicine-overview_en.pdf"

8 BTG International Inc, VORAXAZE-glucarpidase injection, powder, for solution Package insert [revised 2019 Aug; cited 2023 Mar 10]. Available from: "https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=acaef5a6-b740-40e3-8ffe-74a75c74745c"

9 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

10 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

11 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

12 Breithaupt H, Küenzlen E. Pharmacokinetics of methotrexate and 7-hydroxymethotrexate following infusions of high-dose methotrexate. Cancer Treat Rep 1982 Sep;66(9):1733-1741. PMID: 6956440.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the 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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606