medac Asparaginase-Aktivitäts-Test
MAAT

English

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medac Asparaginase-Aktivitäts-Test (MAAT)

A quantitative enzyme assay for the determination of asparaginase activity

Catalogue No.: 550

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

The enzyme L-asparaginase is of cardinal importance in the treatment of acute lymphatic leukaemia (ALL) in children and adults.

The principle of asparaginase therapy is the enzymatic cleavage of the amino acid asparagine into aspartic acid and ammonia, with consequent depletion of asparagine levels in the plasma and cerebrospinal fluid (see also test principle, page 2).

The test kit can be used to measure asparaginase activity during the course of therapy, with the purpose of ensuring that the asparaginase therapy is as good as possible.

The objectives of asparaginase activity monitoring are:

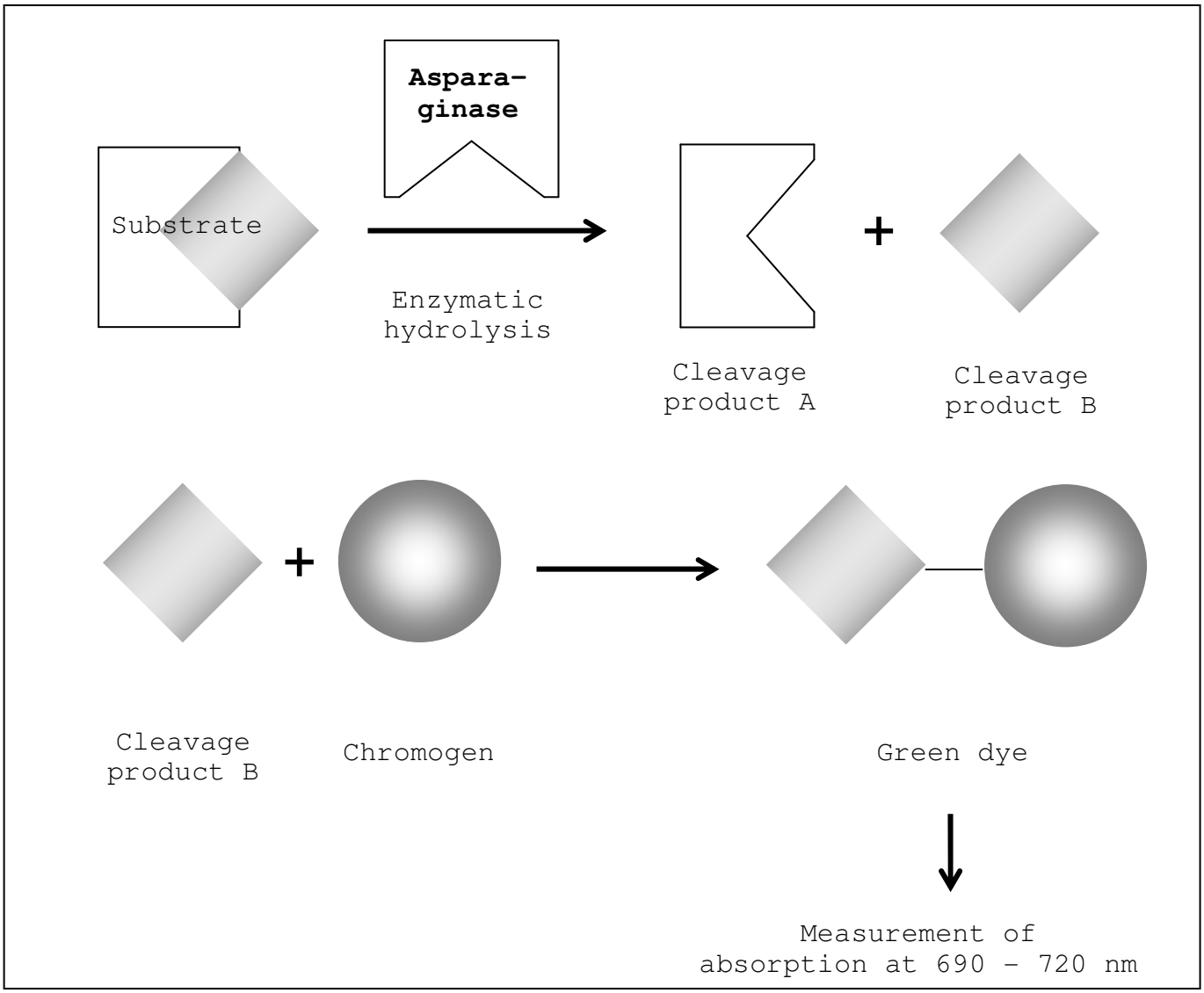
* To measure serum asparaginase activity during therapy as a surrogate parameter for asparagine depletion.

* To detect any „silent inactivation“ evoked by an immune response, with resulting curtailment of the period of activity of the asparaginase preparation.

* To provide a rational basis for deciding whether to change to some different asparaginase preparation or whether to adjust the dose.

The MAAT assay is a homogeneous test for measuring asparaginase activity. It enables the objectives of asparaginase activity monitoring to be fully achieved.

PRINCIPLE OF THE TEST



Asparaginase cleaves an asparagine-analogous substrate into two cleavage products A and B.

The cleavage product B released in this way undergoes a complex reaction with a chromogen, forming a green colour complex which can be detected by absorption measurements.

The optical density is measured at 690 – 720 nm, and the asparaginase activity of the sera is read off from a calibration curve.

ADVANTAGES OF THE TEST

- **Flexible handling by breakable microwell strips**
- **Simple handling because no washing or centrifuging steps**
- **Test duration only about two hours**
- **High precision of the test results with Asparaginase medac®**

KIT CONTENTS:

Cat. no.: 550

1. Microplate: 6 x 8 wells (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, specially treated.
2. Control: 2 vials with 0.5 ml each, Asparaginase medac® from *E. coli* in human serum, lyophilized.
3. Calibrators: 2 vials with 0.5 ml each, Asparaginase medac® from *E. coli* in human serum, lyophilized:
   - 3a. Calibrator 1: 600 U/L
   - 3b. Calibrator 2: 300 U/L
   - 3c. Calibrator 3: 50 U/L
   - 3d. Calibrator 4: 0 U/L
4. Sample diluent: 1 bottle with 110 ml, PBS/TWEEN/BSA, pH 7.2 – 7.4, ready to use, contains ProClin™ 300, Xi Irritant, R 43, S 24-37-60.
5. Substrate: 2 vials with 0.75 ml each, ready to use, contains ProClin™ 300.
6. Chromogen: 2 vials with 1.0 ml each, concentrated, R 52/53.
7. Chromogen diluent: 2 vials with 2.0 ml each, ready to use.

1. STORAGE AND STABILITY

Material/Reagent	Stated	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	until expiry date
Control	opened, reconstituted	2...8 °C	4 weeks
Calibrators	opened, reconstituted	2...8 °C	4 weeks
Sample diluent	opened	2...8 °C	8 weeks
Substrate	opened	2...8 °C	4 weeks
Chromogen	opened	2...8 °C	4 weeks
Chromogen diluent	opened	2...8 °C	4 weeks

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Adjustable micropipettes.

2.2. Clean glass or plastic containers for dilution of specimen and Chromogen.

2.3. Microplate reader with filter for 690 – 720 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate

The aluminium bag has to be tightly resealed together with the desiccant after removal of wells. Storage and stability of the wells are indicated in the table on page 16.

3.2. Calibrators and control

Reconstitute the lyophilized control and calibrators each with 0.5 ml sample diluent. Turn over gently the closed vial to take of the particles adhered to the stopper.

3.3. Chromogen dilution

Mix the Chromogen with the chromogen diluent 1 + 2. 800 µl of the chromogen dilution is needed for 8 wells.

Example:

Number of wells	Chromogen (µl)	Chromogen diluent (µl)	Σ (µl)	Pipetting volume needed (µl)
6	240	480	720	600
7	280	560	840	700
8	320	640	960	800
9	360	720	1080	900
10	400	800	1200	1000
11	440	880	1320	1100
12	480	960	1440	1200
13	520	1040	1560	1300
14	560	1120	1680	1400
15	600	1200	1800	1500
16	640	1280	1920	1600

Comment: The chromogen warms up if mixed with chromogen diluent.

The mixture of chromogen and chromogen diluent must not be stored. It has to be used immediately.

Do not mix reagents from different lots or manufacturers.

Valid and reproducible results are only obtained if the test procedure is precisely followed and test kit-specific reagents are used.

4. SPECIMEN

4.1. The test is suitable for serum samples. The patient samples can be stored for 7 days at 2–8 °C. Long term storage should be performed at ≤ –20 °C. Repeated thawing and freezing of the samples has to be avoided.

4.2. Do not pre-treat sera in any way (e.g. for inactivation). Sera should not be lipemic, not contain any red blood cells and should not be contaminated with micro-organisms.

4.3. Sera have to be diluted 1:10 with sample diluent (e.g. 20 µl serum + 180 µl sample diluent). Samples out off the measuring range can be diluted further.

5.A. TEST PROCEDURE

5.1. Cut open the aluminium bag of the microplate above the zip fastener and take out the required number of wells (see 3.1.).

5.2. Pipette 20 µl each of control, calibrators and samples into the wells of the microplate.

5.3. Add 20 µl of substrate to each well.

Pipetting of calibrators, control, samples and substrate should be finished within 10 min. Shake gently in order to mix all components thorougly. Afterwards the microplate has to be incubated immediately.

5.4. Incubate the covered microplate wells for 60 min (± 2 min) at room temperature (22 °C ± 1 °C).

5.5. Towards the end of the incubation time prepare the chromogen dilution (s. 3.3.).

5.6. Pipette 100 µl of the chromogen dilution into all microplate wells.

5.7. Incubate the covered microplate wells for 60 min (± 2 min) at room temperature (22 C ± 1 C).

Ensure for a good mixing of all components by shaking gently.

Ensure that there are no air bubbles in the wells before photometric reading.

The photometric reading should be done immediately when the final incubation step is completed.

5.B. TABLE OF THE TEST PROCEDURE

	Calibrators	Control	Samples
Calibrators Control Samples	20 µl – –	– 20 µl –	– – 20 µl
Substrate	20 µl	20 µl	20 µl
Incubation 60 min, room temperature (22 ± 1 °C)			
Chromogen dilution	100 µl	100 µl	100 µl
Incubation 60 min, room temperature(22 ± 1 °C)			
Photometric reading at 690 – 720 nm			

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read the OD values at a wave length of 690 – 720 nm. The OD value of the blank is read against air.

* The nominal activity range of the control is printed on the box label.

* Validity Criteria

  - The activity of the **control** has to be within the **nominal range** (see box label).
  - The OD value of **calibrator 1** has to be **> 1.500.**
  - The OD value of **calibrator 4** has to be **< 0.150.**

The run has to be repeated if the results do not meet the specification.

* Calibration curve and quantification

  The OD values of the calibrators are plotted against the activity values. We recommend to use a cubic spline approximation.

  The activity values to the corresponding OD values of the samples can be read from the calibration curve.

The measuring range spans from 30 to 600 U/L. Samples with activities below the measuring range have to be interpreted as < 30 U/L. Samples above the measuring range have to be interpreted as > 600 U/L. These values must not be extrapolated but should be retested in higher dilutions.

If a sample was measured in a higher dilution than 1:10 the activity read from the calibration curve has to be multiplied with the additional dilution factor (e.g., used dilution of the sample 1:40, read activity 250 U/L ⇒ real activity = 250 U/L x 4 = 1000 U/L).

6.B. INTERPRETATION OF RESULTS/LIMITATION OF THE METHOD

* With the medac Asparaginase-Activity-Test (MAAT) the asparaginase activity of asparagine degrading enzymes can be measured. This is valid for the asparaginase activity of all commercially available asparaginase preparations.

* The calibration of the test is performed against Asparaginase medac®, so that samples from patients who are treated with Asparaginase medac® are accurately assayed. The validation had been carried out in comparison with a reference method (AHA test, see Lanvers et al., 2002).

* Because of the dependence of the enzyme activity on the testing method, the measurement of samples which contain other asparaginase preparations may result in systematic deviations of the measured activity. In order to determine this deviation for Oncaspar®, an investigation of samples from patients who had been treated with Oncaspar® had been performed in comparison with the AHA reference method. The results revealed an elevated activity at an average of 24 % (SD = 10 %; N = 134). For quality assurance and monitoring of the intended dose intensity during therapy these normally marginal deviations may be neglected.

* In the present state of scientific knowledge it is not possible to define a clear limit value for asparaginase activity, above which 100 % asparagine depletion is guaranteed. Each user or each study group should themselves define their own limit value depending on the objective of therapy. However, Riccardi et al. (1981) point out that, from an asparaginase activity of 100 U/L or above, total asparagine depletion can be guaranteed, and consequently an activity of 100 U/L can be regarded as the „effective minimal activity“.

* High concentrations of hemoglobin and bilirubin do not influence the results.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. PREVALENCE

102 sera obtained from blood donors were measured during the diagnostic evaluation. The asparaginase activity was generally < 30 U/L.

In addition, 52 sera obtained from children were investigated for asparaginase activity. The values of this population also were generally < 30 U/L.

7.B. PRECISION

Sample	Intra-assay variation				Sample	Inter-assay variation			
	mean OD	SD	CV (%)	n		mean U/L	SD	CV (%)	n
Cal. 1	2.162	0.068	3.1	22	**C**	91.5	3.7	4.0	9
C	0.530	0.006	1.1	22	**No. 3**	38.2	1.7	4.5	9
No. 1	0.254	0.006	2.5	22	**No. 4**	125.5	7.5	6.0	9
No. 2	1.380	0.021	1.5	22	**No. 5**	420.3	33.7	8.0	9

Cal.= calibrator; C = control; No. = serum

7.C. RECOVERY

A mean recovery of 93.6 % (SD = 9.9 %) was calculated adding 3 defined asparaginase activities (Asparaginase medac®) each to 3 different sera.

7.D. DILUTION LINEARITY

The dilution linearity was checked with 10 highly reactive sera which were tested in 4 – 5 different dilutions (each 1:2 diluted further).

	Dil. 1 U/L	Dil. 2 U/L	Dil. 3 U/L	Dil. 4 U/L	Dil. 5 U/L	Mean U/L	SD U/L	CV %
No. 1	1731	1789	1845	1897	–	1815	71	3.9
No. 2	440	446	465	476	–	457	17	3.6
No. 3	1569	1638	1665	1672	–	1636	47	2.9
No. 4	359	357	373	375	–	366	9	2.5
No. 5	373	380	407	404	–	391	17	4.4
No. 6	543	520	546	564	614	557	35	6.3
No. 7	940	945	972	979	–	959	19	2.0
No. 8	8876	8889	9248	8986	9125	9025	160	1.8
No. 9	3609	3775	4025	4273	–	3920	291	7.4
No. 10	1087	1068	1116	1153	–	1106	37	3.4

7.E. HOOK EFFECT

There was no hook effect observed for asparaginase activities up to 20 000 U/L.

7.F. LIMIT OF QUANTITATION (LOQ)

The limit of quantitation is 30 U/L.

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the indicated shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFTY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents) should be handled as potentially infectious and used with all necessary precautions.

R 43: May cause sensitisation by skin contact
R 52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S 24: Avoid contact with skin
S 37: Wear suitable protective gloves
S 60: This material and its container must be disposed of as hazardous waste

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

Date of issue: 01.05.2012

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