MAAT

medac Asparaginase Activity Test

For a reliable Control of Asparagine Depletion by specific Drug Monitoring

medac

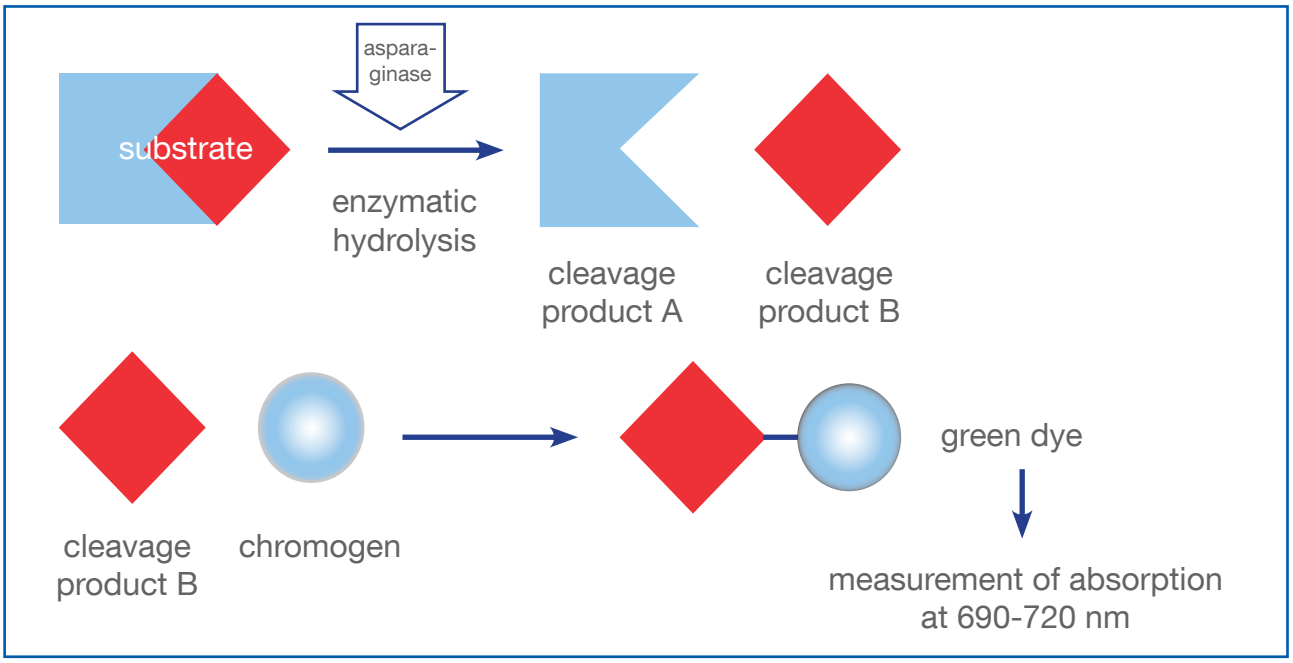
The medac Asparaginase Activity Test offers an Opportunity to optimize Asparaginase Treatment by therapeutic Drug Monitoring

- Monitoring of asparaginase activity can detect silent inactivation caused by the patient‘s immune response.
- Use of an alternative preparation in case silent inactivation is detected.
- Controlling the duration of asparaginase activity during treatment course.
- A target level of ≥100 U/L asparaginase activity is reported to ensure therapeutic benefit [4, 6, 7].
- Determination of the lowest possible dosage of the drug, while retaining full efficacy.
- Rapid availability of exact results for an appropriate therapeutic decision.

Kit Content

- Microplate, 6 x 8 wells (standard 96 well format), breakable, U-form
- 2 vials asparaginase control, app. 100 U/L, 0.5 ml, lyophilized
- 2 vials asparaginase calibrator, 0 U/L, 0.5 ml, lyophilized
- 2 vials asparaginase calibrator, 50 U/L, 0.5 ml, lyophilized
- 2 vials asparaginase calibrator, 300 U/L, 0.5 ml, lyophilized
- 2 vials asparaginase calibrator, 600 U/L, 0.5 ml, lyophilized
- 1 vial sample diluent, 110 ml, ready to use
- 2 vials substrate, 0.75 ml, ready to use
- 2 vials chromogen, 1 ml, concentrate
- 2 vials chromogen diluent, 2 ml, ready-to-use

Test Principle

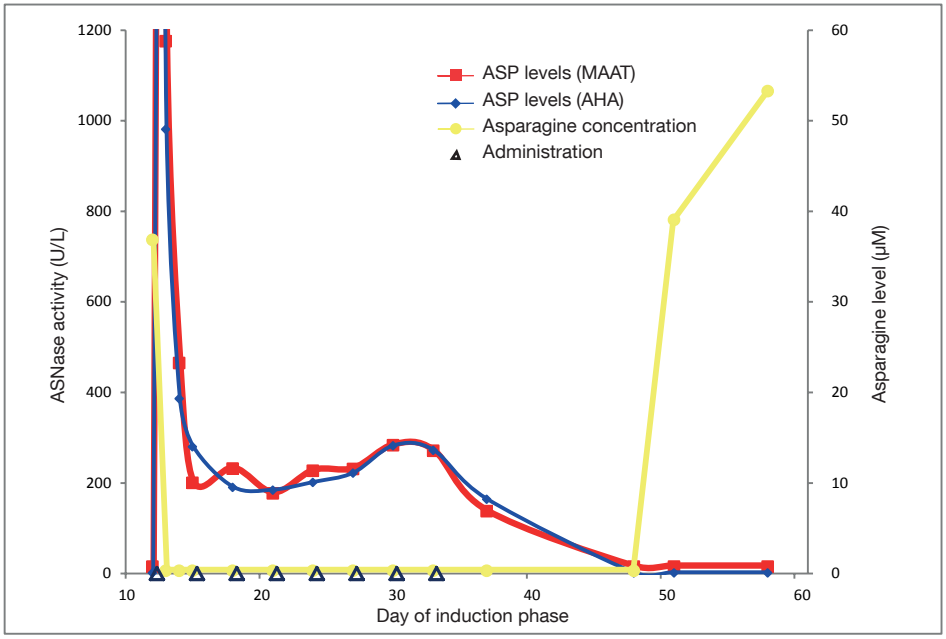


Test Characteristics

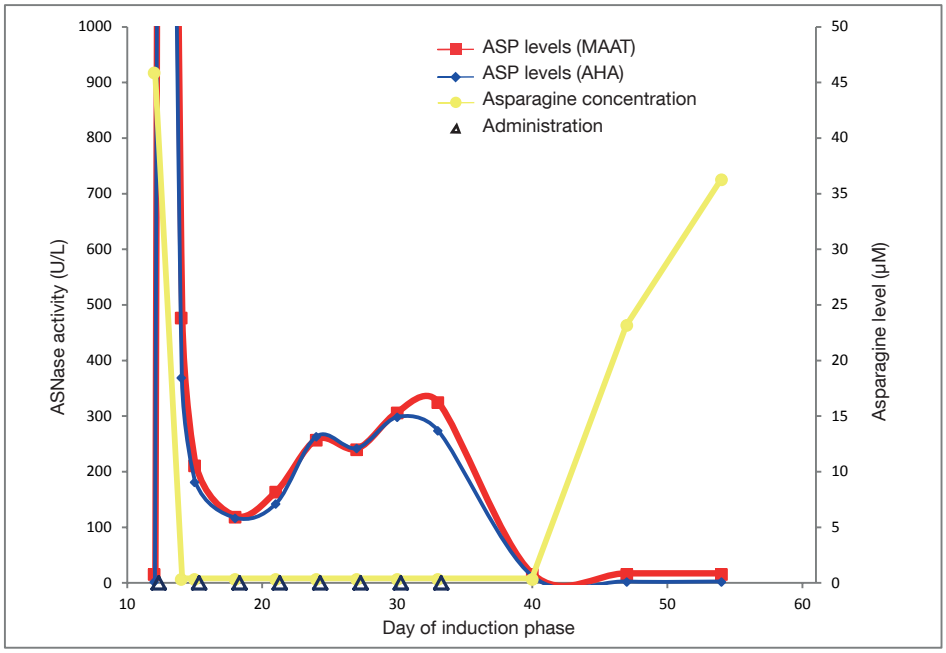
- Stability: 8 weeks after opening the kit
- Analytic range: 30-600 U/L (∞ by further dilution, linearity validated)
- Sample: Serum or EDTA-plasma
- Assay time: appr. 3 h
- Required material not included: Adjustable micropipettes
  Microplate reader with measuring wavelength within 690-720 nm
  Clean containers for dilution of samples and chromogen

Asparaginase Activity during Treatment with different Asparaginase Preparations using different Methods of Monitoring

ALL patient (♂/15 years) under Spectrila® treatment (5000 U/m²) during the first exposure (induction phase)

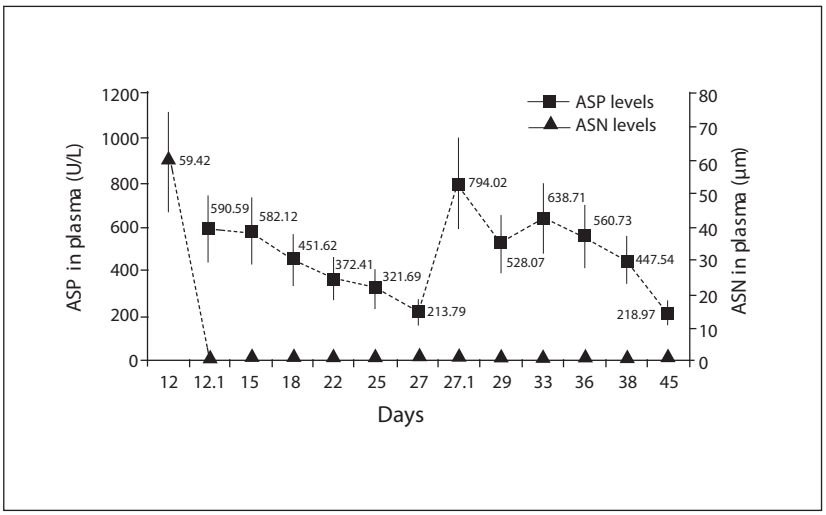


ALL patient (♂/2 years) under Asparaginase medac treatment (5 000 U/m²) during the first exposure (induction phase)

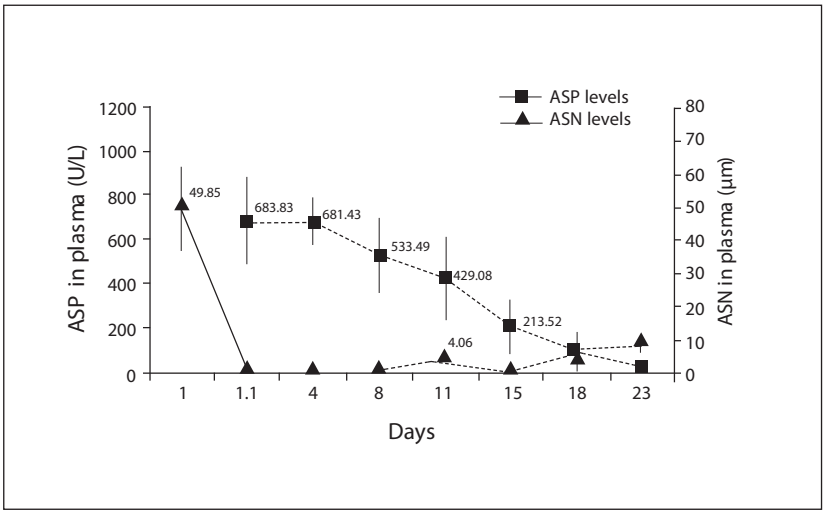


Development of Asparaginase Activity Levels

ALL patients treated with 1000 U/m² Oncaspar® [6]



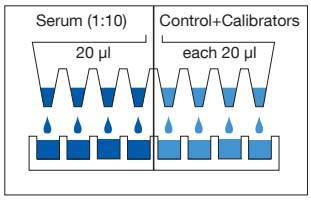
Plasma: asparaginase (ASP) activity levels* (■) vs asparagine (ASN) levels* (▲) during the first exposure (induction, phase IA) to Oncaspar® (given on days 12 and 27). *mean ± SD

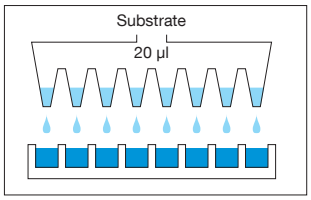


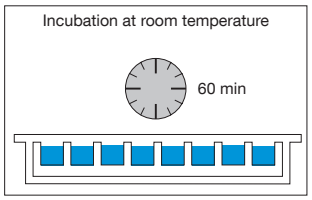
Plasma: asparaginase (ASP) activity levels*(■) vs asparagine (ASN) levels* (▲) during the second exposure (reinduction, protocols II or III) to PEG-ASP (considered to given conventionally given on day 1). *mean ± SD.

- MAAT and AHA method deliver comparable results.
- Asparaginase levels ≥100 U/L correlate with complete asparagine depletion.
- MAAT can be used for various commercially available asparaginase preparations.[3), 6), 8)]

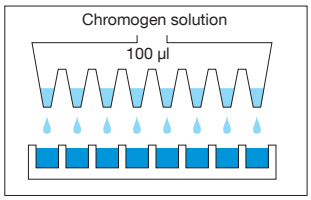
Test Procedure

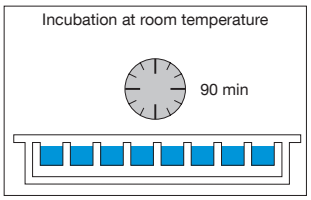


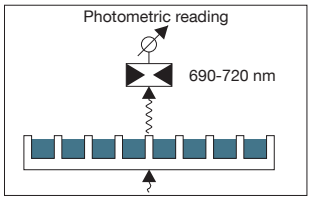




1) Pipette 20 µl serum 1:10 diluted in sample diluent and 20 µl control and calibrators reconstituted with sample diluent.
2) Add 20 µl substrate in all wells and **3)** incubate for 60 minutes at room temperature.
4) Add 100 µl chromogen solution in all wells, **5)** incubate for 90 minutes at room temperature and
6) read the OD values at 690-720 nm.







Performance Characteristics

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 were generally < 30 U/L as well.

Precision

Intraassay Variation determined in 4 reactive samples between 30-600 U/L, 22 fold measurement:

Sample	U/L	Mean OD	CV (%)
Calibrator	600.0	2.162	3.1
Control	96.4	0.530	1.2
Serum A	39.6	0.254	2.5
Serum B	310.3	1.380	1.5

Interassay Variation determined in 4 samples in 9 test runs, independent from each other:

Sample	Mean U/L	CV (%)
Control	91.5	4.1
Serum No. 1	38.2	4.5
Serum No. 2	420.3	8.0
Serum No. 3	125.5	6.0

Recovery

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

Dilution Linearity of reactive Sera

The dilution linearity was checked with highly reactive sera which were tested in 4-5 different dilutions (1:2 serial dilution) and showed a CV of 1-7 %.

	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

There was no hook effect observed for asparaginase activities up to 20000 U/L.

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Advantages of MAAT

MAAT is used as a clinical trial assay by several study groups in different trials worldwide (e.g. AIEOP-BFM ALL 2009; IntReALL 2010[3]; 58081 EORTC[3), 8)]).

- Identification of patients with silent inactivation of asparaginase
- Suitable for measuring activity of various asparaginase preparations
- Optimizing of individual asparaginase dosing
- Drug monitoring at point of care
- Results available in 3 h
- High accuracy
- Easy-to-use: no washing or centrifugation steps

References

1) Boos et al.: Monitoring of Asparaginase Activity and Asparagine Levels in Children on Different Asparaginase Preparations. European Journal of Cancer. 32A (9), 1544-1559 (1996).

2) Lanvers et al.: Analytical validation of a microplate reader-based method for the therapeutic drug monitoring of L-asparaginase in human serum. Analytical Biochemistry. 309, 117-126 (2002).

3) Leão and Costa: Therapeutic Drug Monitoring (TDM) of Asparaginase – the Porto Experience. Paper presented at The Children's Leukemia Group EORTC meeting; 2014 Oct 10-11; Porto, Portugal.

4) Mueller et al.: Pegylated Asparaginase (Oncaspar) in Children with ALL: Drug monitoring in reinduction according to the ALL/NHL-BFM protocols. British Journal of Hematology. 110, 379-384 (2000).

5) Riccardi et al.: L-Asparaginase pharmacokinetic and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Research. 41, 4554-4558 (1981).

6) Rizzari et al.: A pharmacological study on pegylated Asparaginase used in front-line treatment of children with acute lymphoblastic leukemia. Haematologica/The Hematology Journal. 91(1), 24-31 (2006).

7) van der Sluis et al.: Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. Haematologica. 101(3), 279-285 (2016).

8) Costa: Therapeutic Drug Monitoring (TDM) of Asparaginase – the Porto Experience. Poster session presented at the 10th Biennial Childhood Leukemia Symposium, 2016, Apr 25-26, Megaron, Athens, Greece.

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