BIOTINIDASE SERUM/PLASMA

Colorimetric determination on micro plate of Biotinidase in serum and plasma

CLINICAL SIGNIFICANCE

Biotinidase (BTD) is an enzyme present in pancreatic juice that serves to release biotin in the intestinal lumen by splitting the biotin-laxin bond.
In the presence of biotinidase deficiency, the biotin requirement increases, and must be supplemented by the permanent administration of pharmacological doses.

TEST SUMMARY

Para-biotinyl aminobenzoic acid is hydrolyzed by biotinidase into biotin and para-aminobenzoic acid; the latter, in an acid environment, reacts with ammonium sulphamate, NEDD and nitrite to form a pink-purple coloured compound.

SAMPLES

Serum, plasma citrate, heparinate or EDTA.
Serum/plasma stability 7 days at -20°C.

REAGENTS

Reagent 1: Phosphate buffer pH 6.0, EDTA 0.2 g/l, stabilizers and preservatives.

Reagent 2: Phosphate buffer pH 6.0, EDTA 0.2 g/l, enzyme inhibitor, stabilizers and preservatives.

Reagent 3: Blocking solution, 1% sulphuric acid.

Reagent 4: Sodium nitrite 1 g/l.

Reagent 5: Ammonium sulphamate 5 g/l.

Reagent 6: NEDD 1 g/l.

Standard: pABA at different concentrations; stabilizers and preservatives (0.8 - 0.6 - 0.4 - 0.3 - 0.2 - 0.1 mM)

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS equipped with temperature control and stirring.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

The reagents are ready to use and stable, stored at 2-8°C, until the expiry date indicated on the package. Be careful not to contaminate the reagents once the bottles have been opened.
Bring the Reagents to working temperature before use.

PROCEDURE

Kind of analysis:	End point
Reading time:	10 minutes
Wavelength:	546 nm
Temperature:	37°C
Zero:	Reagent Blank

Reagents	Reagent Blank	Standard	Sample	Sample Blank
Reagent 1	60 µl	120 µl	60 µl	120 µl
Reagent 2	60 µl	--	60 µl	--
H2O distilled	5 µl	--	--	--
Standard	--	5 µl	--	--
Sample	--	--	5 µl	5 µl
Incubate at 37°C for **60 minutes exactly**.				
Reagent 3	40 µl	40 µl	40 µl	40 µl
Reagent 4	20 µl	20 µl	20 µl	20 µl
Reagent 5	20 µl	20 µl	20 µl	20 µl
Reagent 6	20 µl	20 µl	20 µl	20 µl

The volumes in the wells are not the same, but reading vertically, this has no influence.
Mix, incubate for 10 minutes at 37°C and read, against Reagent Blank, the absorbances of the Sample, Sample Blank and Standards.

CALCULATION

Serum/Plasma
For each sample, calculate the difference between the absorbance of the Sample and that of the corresponding Sample Blank.
(Δabs = AbsSample – AbsSampleBlank)

Prepare a calibration curve with the Absorbances of the Standards and their concentrations of pABA (mM).

Interpolate the Δabs of the previously calculated samples on the calibration curve to determine the concentrations of pABA (mM) relative to the samples.

Biotinidase activity (nmol/min/ml) or (U/L)

$$\frac{\text{pABA sample (mM extrapolated)}}{\text{60 (incubation minutes)}} \times 1000$$

REFERENCE RANGES

SERUM PLASMA	nmol/min/ml
Normal	4.4 – 12.0
Obligatory heterozygotes	2.2 – 5.2
Partial deficit	0.7 – 2.1
Deficiency	< 0.7

Every laboratory should establish own reference intervals in accordance with own population.

NOTE

- As with any diagnostic procedure, if the results are incompatible with the clinical presentation, the doctor should evaluate the data obtained using this test in light of other clinical information.
- For IVD use.

CALIBRATION/ QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control plasma on human base are available on request:

SN00830 **1 x 1 ml (lyophile)**
Control Plasma

TEST PERFORMANCE

Precision

Intra-assay (n = 5)	Mean	SD	CV%
Sample 1	1,64	0,22	13,63
Sample 2	1,47	0,35	24,11
Sample 3	8,10	0,58	7,16
Sample 4	5,57	0,45	8,03
Sample 5	2,73	0,34	12,41

Inter-assay (n = 6)	Mean	SD	CV%
Sample 1	3,65	0,38	10,50
Sample 2	7,5	0,83	11,12
Sample 3	5,4	0,72	13,41
Sample 4	11,18	1,28	11,44
Sample 5	1,13	0,28	24,75

Sensivity / limit of detection

Limit of quantification (LOQ): 0.016 mM = 0.26 U/L.
(CV% <20)

Detection Limit (LOD): 0.005 mM = 0.08 U/L.

Interferences
There are few drugs reported in the literature that may interfere in the determination of BTD: VPA and Sulfonamides [2].
Hemoglobin, bilirubin and lipids at high concentrations can interfere in spectrophotometric evaluation [3]. In case of strongly hemolyzed and/or lipemic and/or icteric sample, we suggest reporting it on the report.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE SN00800	(100 TESTS)
Reagent 1	1 x 20 ml (liquid)
Reagent 2	1 x 7 ml (liquid)
Reagent 3	1 x 10 ml (liquid)
Reagent 4	1 x 5 ml (liquid)
Reagent 5	1 x 5 ml (liquid)
Reagent 6	1 x 5 ml (liquid)
Standard 0.1 mM	1 x 2 ml (liquid)
Standard 0.2 mM	1 x 2 ml (liquid)
Standard 0.3 mM	1 x 2 ml (liquid)
Standard 0.4 mM	1 x 2 ml (liquid)
Standard 0.6 mM	1 x 2 ml (liquid)
Standard 0.8 mM	1 x 2 ml (liquid)

REFERENCES

1. D. A. Dove Pettit and Barry Wolf (1991) Quantitative colorimetric assay of biotinidase activity. Technique in diagnostic human biochemical genetics: a laboratory manual.
2. K.H.Shulpis et al "Low Serum Biotinade Activity in Children with Valproic Acid Monotherapy" - Blackwell Science 2001.
3. M.Morandini "Criteri di Qualità per l'accettabilità dei campioni" RIMel/IjLaM 2006.
4. Cowan, Tina M., Miriam G. Blitzer, and Barry Wolf. "Technical standards and guidelines for the diagnosis of biotinidase deficiency." Genetics in Medicine 12.7 (2010): 464.
5. Strovel, Erin T., et al. "Laboratory diagnosis of biotinidase deficiency, 2017 update: a technical standard and guideline of the American College of Medical Genetics and Genomics." Genetics in Medicine 19.10 (2017): 1079.

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SYMBOLS

IVD Only for IVD use
LOT Lot of manufacturing
REF Code number
Storage temperature interval
Expiration date
Warning, read enclosed documents
Read the directions
Biological risk

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