Tryptase-ELISA

ELISA for the quantitative determination of total Tryptase in human Serum or Plasma

The total Tryptase level in human blood indicates the number and activity of mast cells. These play a key role in allergic reactions and increase quantitatively under inflamation conditions. When mast cells are activated, they release a number of mediators, which can lead to signs and symptoms of allergic reactions. To this released mediators belongs, among others, the Tryptase. Total Tryptase can be differentiated in two isoforms of α-Tryptase and three isoforms of ß-Tryptase. In contrast to ß-Tryptase, which is stored in the granula of mast cells until mast cell degranulation, the α-Tryptase is released continuously from the mast cells (baseline level). Therefor a transient increase in the level of Tryptase can be an indicator for allergic reactions. A persistent elevated baseline level of Tryptase indicates an increased number of activated mast cells and is linked with the disease of mastocytosis.

The Tryptase-ELISA is intended for the quantitative determination of total Tryptase in human serum or plasma.

Increased Tryptase levels can be used for the evaluation of risks for allergic reactions. Total Tryptase is also used as WHO diagnostic criterion for mastocytosis.

The Trypatse-ELISA is determined for the application by qualified and trained professionals.

Tryptase-ELISA Specifications

- Risk assesment for severe allergic reactions
- WHO diagnostic criterion for mastocytosis
- Determination of the total Tryptase level in human blood
- Excellent sensitivity and specificity

REF 25050 ▽Σ 96 Determinations

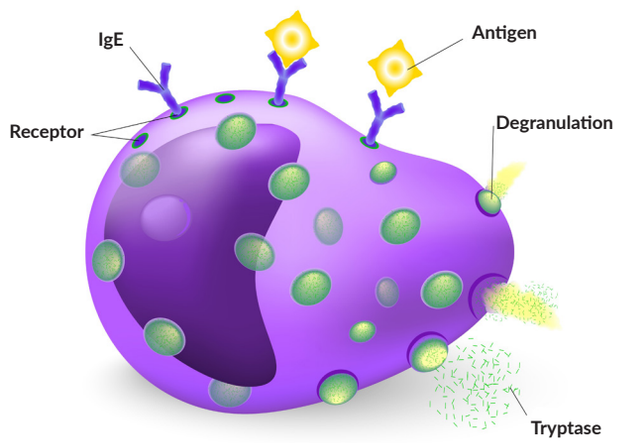

Figure 1 Mast Cell

Expected values

In healthy individuals the Tryptase baseline levels have been determined between 1-15 µg/L. Every human has an own individual baseline level, which is usually consistant over the time elapsed. In humans with increased Tryptase baseline level, > 10 µg/ml, a higher risk for anaphylactic reactions is seen.

In allergic reactions tryptase is released into the blood in the context of mast cell activation. Peak values are reached within 30-90 minutes after the start of the allergic reaction. After that the values start to fall within 3-6 hours (biological half-life amounts 2 hours). 24 hour later the base level should be reached. If the value is still increased, the baseline level should be tested a few days later.

For the interpretation of the tryptase value it is important to consider in which situation the value was measured and at what point of time the measurement took place.

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Performance data

Serum samples (n=24) were taken from the serum bank at Dr. Fooke Laboratorien and tested for Tryptase by the newly developed Sandwich ELISA system (Dr. Fooke Laboratorien). Also 43 serum samples from the university hospital in Ulm, Germany, were used for the evaluation of the Tryptase-ELISA. The results were compared to an established in-vitro method for the determination of Total-Tryptase (ImmunoCAP® Tryptase, ThermoScientific).

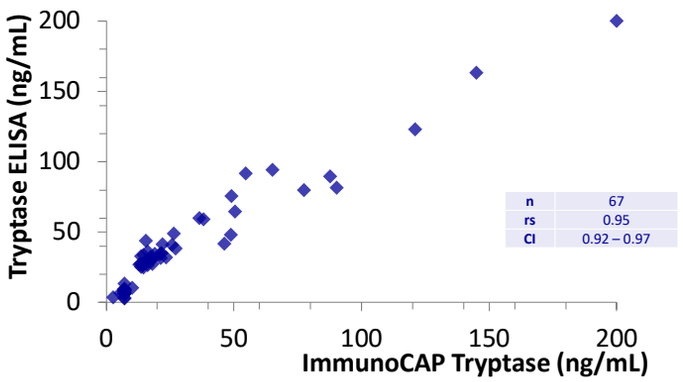

Figure 2
Spearman correlation between Tryptase ELISA (Dr. Fooke Laboratorien) and ImmunoCAP® Tryptase (ThermoScientific) with n=67 results.

Agreement between the newly developed Tryptase-ELISA and ImmunoCAP® Tryptase, according to Spearman were found at 0.95 for 67 serum samples. Inter- and intra-assay variations and precision of the Tryptase ELISA from Dr. Fooke Laboratorien were observed at < 10 %.

A) Standard curve

Standard (ng/mL)	Intra-Assay Variation (%)	Inter-Assay Variation (%)
200	4,15%	3,62%
100	3,18%	4,66%
50	2,81%	5,32%
25	4,91%	5,03%
12,5	5,27%	8,04%
5	4,37%	4,18%
2	5,40%	8,48%
1	5,95%	6,44%

B) Serum sample

Serum-probe	Intra-Assay Variation (%)	Inter-Assay Variation (%)
#15	2,49%	2,78%
#2	6,92%	7,58%
#6	2,08%	2,24%
#1	0,77%	1,08%
#4	2,88%	3,68%
17-1805	1,80%	1,68%
17-1803	2,33%	2,94%

C) Precision

Serum-probe	ImmunoCAP (ng/mL)	Tryptase ELISA (ng/mL)	Präzision (%)
18-3200	15,5	14,94	96,25
#25	26,3	24,70	93,52
#32	30,4	30,81	98,67
#38	50,4	52,46	96,07

Figure 3
A) Inter- and intra-assay variations of the standard curve
B) Inter- and intra-assay variations of serum samples
C) Precision between Tryptase ELISA (Dr. Fooke Laboratorien) and ImmunoCAP® Tryptase (ThermoScientific), n=4 results.

Area under the curve (AUC) value was found at 0.99 compared to ImmunoCAP® Tryptase. A sensitivity and specificity of 0.96 and 1.00 were observed.

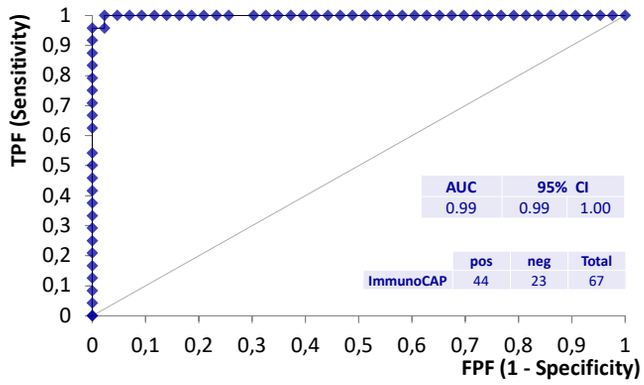

Figure 4
ROC analysis for Tryptase ELISA (Dr. Fooke Laboratorien) vs. ImmunoCAP® Tryptase (ThermoScientific) with n=67 results.

The results of the Tryptase ELISA, performed manually and with a fully automated system, are comparable.

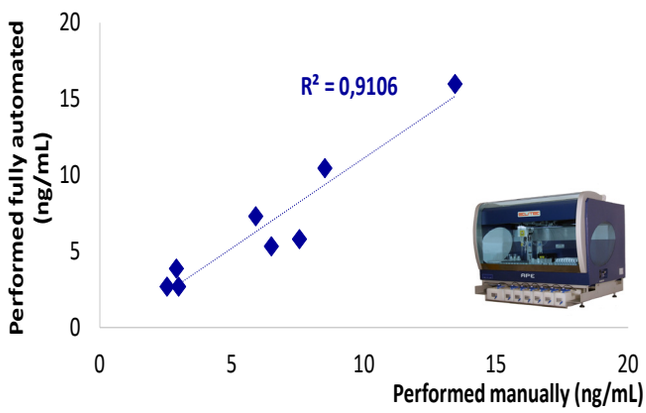

Figure 5
Spearman correlation between Tryptase ELISA (Dr. Fooke Laboratorien) performed manually and fully automated n=8 results.

Literature

1. Hogan AD & Schwartz LB (1997) Markers of mast cell degranulation. Methods 13(1):43-52.
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4. Schwartz LB (1985) Monoclonal antibodies against human mast cell tryptase demonstrate shared antigenic sites on subunits of tryptase and selective localization of the enzyme to mast cells. Journal of immunology 134(1):526-531.

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