IDK® Tryptase

ELISA for the determination of total tryptase in serum

Diagnostics of mastocytosis and other mast cell diseases with elevated basal tryptase

- mastocytosis
- insect bite allergy
- hematological neoplasms
- myocardial infarction
- heroin abuse

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Overview:

What is tryptase?

Tryptase is an enzyme of mast cells that after activation is released by degranulation. There are several tryptase forms (α, β, γ and δ), which are produced and released by mast cells.

The concentration of total tryptase in serum, i.e. all tryptase forms, correlates with the number of mast cells in the bloodstream.

Why should you determinate total tryptase in the serum?

Total tryptase should be used to diagnose **anaphylaxis**, as a diagnostic criterion for **mastocytosis** or for the detection of of a **mast cell activation syndrome**.

When should it be tested?

- for symptoms of anaphylaxis: **Pruritus, erythema, anxiety, dyspnea, hypotension, convulsive abdominal pain, vomiting**
- for **allergy sufferers with severe episodes**
- in case of suspicion of **mastocytosis** (systemic or cutaneous) or **mast cell activation syndrome**
- to exclude anaphylaxis as a possible cause of death

Which sample material is required?

Serum is required. No further sample preparations are necessary. Several tests per patient are useful in order to determine the basal value.

How long does the *IDK*® test take?

100 µl 1:5 diluted serum is needed.
The procedure lasts about 2.5 hours.

➲ **In acute situations the sample should be analyzed as soon as possible after onset of symptomatology, in order to avoid life-threatening conditions.**

Anaphylaxis

Anaphylaxis was first described in 1901 by Portier and Richet[1]. In their classical form, the skin, the respiratory organs as well as the cardiovascular and gastrointestinal systems are affected. The interpretation can be very variable in the individual case and is thus difficult to diagnose. This is made even more complicated by the fact that a uniform definition has so far been lacking.
For Germany the incidence is currently estimated at 1–2% of the total population[2].

The penetration of allergens through natural barriers (skin, gastrointestinal tract) leads to a contact with mast cells and basophils. If these are sensitized, mediators are secreted which, in turn, leads to reactions in different organs and tissues and in the most severe course appear as symptoms of anaphylaxis.

Clinical criteria for anaphylaxis

At a physician's conference in 2005 [3], a recommendation was made for the definition of anaphylaxis based on clinical signs and published as a guideline in 2015 [4]:

Anaphylaxis is likely to occur if one of the following three criteria is met:

1. acute onset (minutes to hours), taking into account the
   a. skin/mucosa (nettle rash, generalized itching) redness, swelling of the lips/tongue/palate cones) and the respiratory tract (dyspnea, wheezing/bronchospasm, whistles, reduced expiratory peak flow) or
   b. reduced blood pressure and associated symptoms (hypotension, syncope)
2. two or more criteria after exposure to a known allergen (minutes to hours)
   a. Severe allergic reaction in the anamnesis
   b. Skin/mucosal manifestation (hives, generalized itchiness/rotations, swelling of the lips/tongue/palatal cones)
   c. airways (dyspnea, wheezing/bronchospasm, whistling, reduced expiratory peak flow)
   d. reduced blood pressure and associated symptoms (hypotension, syncope)
   e. Food allergies: gastrointestinal symptoms (convulsive abdominal pain, vomiting)
3. low systolic blood pressure following exposure to a known allergen (minutes to hours) or a drop in blood pressure by 30% of normal value [3, 4].

Age	Systolic blood pressure
1 month – 1 year	< 50 mm Hg
1– 5 years	< 60 mm Hg
6– 14 years	< 60 mm Hg
>14 years	< 70 mm Hg
Adults	< 100 mm Hg

Systolic blood pressure – age-dependant normal values
Source: Werner-Busse et al. (2014)

Occurrence, severity and risk factors of anaphylaxis

Anaphylaxis often occurs in the following situations:

- taking **medication**, in particular parenteral administration of antibiotics, painkillers, anaesthetics or X-ray contrast media
- consumption of certain **foodstuffs**, often peanuts, nuts, seafood, eggs, milk, and soy beans
- after administration of pollen extracts, e.g. for hyposensitization against hay fever (subcutaneous immunotherapy SCIT). In particular, the stationary rapid or rush hyposensitization against insect toxins entails a high risk of anaphylactic reaction.
- **insect bites, scorpion bites, contact with snake venom** (Botox in creams) or plant secretions (latex in rubber gloves)
- after administration of **antiserum and other blood products** [4]

The **occurrence and severity of anaphylaxis** depends on personal risk factors. That are for instance the intake of drugs, such as anti-hypertensives (ACE inhibitors and β blockers) and painkillers (acetylsalicylic acid and COX inhibitors), **existing underlying diseases** (mastocytosis, allergic asthma and increased total basal tryptase in serum) and **life-style** (sports, consumption of alcohol, drugs and infections). **Men** are affected more by anaphylaxis than women [4].

The extent of the anaphylaxis will be indicated in four degrees [5]:

Grade I:	sneezing, coughing, wheals, itching, redness of the skin, oedema, tachycardia
Grade II:	anxiety, dyspnea, abdominal cramps, congested neck veins, blood pressure drop
Grade III:	severe shortness of breath, seizure trap, leeching, strong drop in blood pressure
Grade IV:	pale or livid skin, loss of consciousness, pulse not palpable

Diagnostics of anaphylaxis

In the acute phase of anaphylaxis it is recommended to

- determine total tryptase in serum (serum tryptase)
- exclude life-threatening cardiac diseases by means of ECG, and
- carry out an extensive anamnesis [2, 4, 6, 7].

Mast cells, tryptase and anaphylaxis

Mast cells are large lymphatic cells that are formed in the bone marrow and in the entire organism, especially in the skin, intestines and lungs. They bear granules containing, among others, tryptase and histamine.

Tryptase occurs in various forms: Two α- (αI and αII) and three β-tryptases (βI, βII and βIII). The α-tryptases have an almost 90% sequence homology to the β tryptases. Both forms of tryptase are produced by mast cells and constantly released [8]. The concentration of total tryptase in the blood reflects the number of mast cells. Therefore they are used as a measure for mast cell proliferation under certain pathological conditions. For healthy persons their concentration is normally low (0–15 ng/ml)[9].

When mast cells are activated, they release the mediators tryptase and histamine from their granules. The increase in the total tryptase in serum increases after onset of anaphylaxis and reaches its peak about 3–6 hours after exposure. Normal values return after several hours to a few days [9].

„Serum mast cell tryptase levels may help the retrospective diagnosis of anaphylaxis: appropriate blood samples should be sent for analysis."

The Association of Anaesthetists of Great Britain and Ireland (2009)[6]

This opens up the possibility of diagnosing systemic **anaphylaxis**: The Association of Anesthetists of Great Britain and Ireland (s. information box) recommends the determination of serum tryptase in anaphylactic reactions and, for that purpose to take three blood samples:

1. immediately (e.g. after resuscitation),
2. 1 hour after onset of reaction,
3. 24 hours later to obtain baseline value[6].

The level of the serum tryptase value at the peak in relation to the baseline value provides a high specificity and sensitivity[10].

Cut-Off	Sensitivity	Specificity	Probability ratio	
			Positive	Negative
Peak Tryptase > 11,4 ng/ml	0.17	1.00		0.83
Peak Tryptase > 2,99 ng/ml	0.50	0.85	3.33	0.59
Delta Tryptase > 0,8 ng/ml	0.83	0.93	11.86	0.18
Tryptase-Ratio > 1,5 ng/ml	**0.92**	**0.96**	**23**	**0.08**

Sensitivity, specificity, positive and negative probability ratios of peak tryptase, delta tryptase and tryptase ratio[10].

IDK® Tryptase

IDK® Tryptase is an ELISA for the in vitro determination of total tryptase in serum (serum tryptase). It is used to clarify an anaphylaxis of different genesis as well as one criterion for the diagnosis of the mastocytosis and mast cell activation syndrome..

Sample material
Serum is required (100 µL, dilution 1:5).

Duration of the test
The test is a 2-step ELISA. Including sample preparation, incubation time and measurement approximately 2.5 hours are needed for the test procedure.

Test characteristics
Measuring range: 1.2–20 ng/mL
Limit of detection: 1.2 ng/mL

IDK® Tryptase	
Sample volume	100 µL
Matrix	Serum
Test principle	ELISA
Cat. No.	K 6814

Tryptase values [11]

Normal value:	0–11,4 ng/mL
Increased basal value:	from 8–10 ng/mL
Skin mastocytosis (CM):	up to 20 ng/mL
Systemic mastocytosis (SM):	> 20 ng/mL

➲ **Samples with elevated tryptase concentrations must be further diluted and measured again.**

Causes of increased basal serum tryptase

Increased basal tryptase concentrations serve as clinical markers for diseases with pathological mast cell activation and associated elevated basal serum tryptase levels.

Mastocytosis
Most patients with elevated basal tryptase concentrations have mastocytosis. Patients with mastocytosis have a 30-fold increased risk of anaphylaxis. The risk of systemic mastocytosis is higher than that of cutaneous mastocytosis. The basal value of the total tryptase in the serum is decisive for the severity of an occurring anaphylactic reaction [11].
However, a singular increase in serum tryptase level alone is not sufficient to diagnose mastocytosis. Elevated serum tryptase concentration over a longer period is to be regarded as a sign of mast cell disease, and further diagnostic tests must be carried out [11]. To make a final diagnosis, several criteria must be met, which are described elsewhere [14].

Insect bite allergy
Frequent triggers for severe and rapidly developing anaphylaxis are insect bites (bees, wasps, bumble bees, etc.)[12]. Therefore , basal serum tryptase determination is recommended in patients with systemic immediate type allergic reactions to insect bite. Above a value of 8–10 ng/ml there exists an increased risk [13].

Hematological neoplasms
Increased serum tryptase levels were found in patients with advanced chronic myeloid leukemia (CML). Compared to patients with normal tryptase concentrations, these patients had a higher progression and event rate. Tryptase appears to be a strong prognostic marker for the progression of CML [15].

Myocardial infarction
High serum tryptase concentrations in patients with acute myocardial infarction with ST elevation (STEMI) following percutaneous coronary intervention (PCI) are associated with poor myocardial perfusion and reduced cardiac function [16].

Heroin abuse
One cause of death after heroin abuse seems to be an anaphylactic reaction to heroin. At *post-mortem*-investigations of heroin victim revealed an increased serum tryptase concentration. Increased values of serum tryptase are often associated with heroin-associated deaths [17].

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