**ImmunoCAP™ Tryptase**

*Is it anaphylaxis?*
Introduction

Hospital admissions for anaphylaxis have increased seven fold over the last decade according to Department of Health figures\(^1\). Anaphylaxis can present with a variety of symptoms including cardiovascular collapse, erythema, bronchospasm, facial swelling or urticaria. However, patients often do not present with all symptoms, making diagnosis difficult. The recommended approach is to stabilise the patient immediately, and to take a series of blood samples to allow retrospective confirmation of an anaphylactic event. Where there is a confirmed anaphylaxis or a strong suspicion, it is important to refer the patient to an allergy clinic for full investigation.

IgE mediated anaphylactic events result in the systemic activation of mast cells, which release cell mediators such as histamine and tryptase. Tryptase levels usually peak within an hour and have an \textit{in vivo} half-life of 2.5 hours, returning to normal within 12-24 hours\(^2,3\) after release. Measurement of tryptase levels has been used over many years to confirm mast cell tryptase activation, which supports a diagnosis of anaphylaxis. The tryptase released on allergic activation with mast-cell degranulation is \(\beta\)-tryptase. In systemic mastocytosis, \(\alpha\)-tryptase levels are continuously elevated due to the large number of mast cells. Measurement at the initial peak and showing reduction to low levels over 24 hours following a reaction eliminates this possible confusion.

Tryptase assay

Total (\(\alpha\) plus \(\beta\)) tryptase can be measured on ImmunoCAP 100, an instrument used in many laboratories for allergy and autoimmunity testing. ImmunoCAP methodology uses fluorescent enzyme immunoassay and a unique solid phase to ensure optimal sites for binding of antibody. All processing steps – sample and reagent handling, incubation, washing and measurement of fluorescence are fully automated, which ensures excellent reliability and reproducibility. The measuring range for an undiluted sample is 1-200ug/l.

Principle of test

Anti-tryptase, covalently bound to ImmunoCAP, reacts with tryptase in the patient’s sample. After washing, enzyme-labelled antibodies against tryptase are added to form a complex. After incubation, unbound enzyme-anti-tryptase is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence in the eluate is measured. To evaluate the test results, the response for the patient’s samples are compared to the concentrations used for the calibration curve, and the results are calculated automatically.
**Clinical uses**

*Anaphylaxis*

* Asthma deaths

*Post mortem diagnosis*

*Mastocytosis*

**Collapse with no obvious cause (may present to A&E first)**

**Prior to bee/wasp immunotherapy***

* Determination of baseline tryptase is important to exclude mastocytosis, because elevated baseline levels are a risk factor for a severe anaphylactic reaction on re-exposure. Studies have shown that mastocytosis is a difficult diagnosis, which may be missed, and as these patients may not be suitable for allergen desensitisation, alternative long-term strategies may be required. In some centres, a baseline tryptase level is a routine precautionary test before a patient starts bee / wasp immunotherapy.

**Sample tubes**

Blood can be collected into plain tube, (EDTA or heparin tubes may also be used). Send samples to laboratory as soon as possible.

**Timing of blood samples**

- 1st sample - once the patient is stabilising *(ideally within one hour)*
- 2nd sample between 3-6 hours
- 3rd sample at least 24 hours post event to exclude mastocytosis

It is essential to record the time that the samples were taken after the anaphylactic event on each tube to allow interpretation.

**Post-mortem samples**

Can be useful to support a diagnosis of anaphylaxis. After death there is no metabolic activity and tryptase levels are very stable, allowing samples to be taken up to 24 hours after death.

When taking a blood sample from a line, where the patient has had multiple infusions, it may be necessary to discard the first 5ml of blood, to reduce the likelihood of haemodilution.

Routine post-mortem testing for tryptase is recommended by the Royal College of Pathologists as part of investigation following asthmatic death.

**Sample storage**

Send the samples to the laboratory, where they will be separated and stored at 4°C for testing. (If testing will not be done within 5 days, storage should be at −20°C. If testing is done in another laboratory, send samples by first class post).
At what level should tryptase be considered to be elevated?

A study\textsuperscript{10} of 126 apparently healthy children and adults (61 males and 65 females), without evidence of mast cell stimulation, was performed. The age range was 12-59 for males and 13-61 for females.

The following results were obtained:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean</td>
<td>3.8ug/l</td>
</tr>
<tr>
<td>95 upper percentile</td>
<td>11.4ug/l</td>
</tr>
</tbody>
</table>

The distribution is shown in figure.

Issues to consider when interpreting results

1. Timescale of tryptase measurements. Tryptase has a relatively short half-life (2.5 hours) \textit{in vivo}; by taking a series of samples over a period of time it is possible to see the level increasing, then returning to normal after 24 hours.

2. Haemodilution – if the patient received multiple infusions an element of plasma dilution will be present which would make the result positively low.

3. Mastocytosis – if the tryptase level is still high after 24 hours, it may be that the patient has mastocytosis, or is still in contact with the allergen e.g. antibiotic administered via a catheter, which is still \textit{in situ}.

4. When a patient arrives at A&E with possible anaphylaxis, it is very important to determine the exact time when the symptoms started.

5. Raised tryptase levels followed by a fall to normal, allow confirmation of an immune response, and distinguish the diagnosis from cardiogenic or vasovagal shock\textsuperscript{11}.

If you can’t find a laboratory offering tryptase testing

If you have any difficulty finding a laboratory to test the sample for tryptase, please send an email to diag.eu@diagnostics.com giving details of your location and we will advise you of the nearest testing laboratory.
Useful allergen-specific IgE ImmunoCAP available for investigation of anaphylaxis

Common allergens implicated in anaphylaxis:

<table>
<thead>
<tr>
<th>ImmunoCAP Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>c1</td>
<td>penicilloyl G</td>
</tr>
<tr>
<td>c2</td>
<td>penicilloyl V</td>
</tr>
<tr>
<td>c5</td>
<td>ampicilloyl</td>
</tr>
<tr>
<td>c6</td>
<td>amoxicilloyl</td>
</tr>
<tr>
<td>c7</td>
<td>cefaclor</td>
</tr>
<tr>
<td>c74</td>
<td>gelatin bovine</td>
</tr>
<tr>
<td>c202</td>
<td>suxamethonium</td>
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<td>k82</td>
<td>latex</td>
</tr>
<tr>
<td>f13</td>
<td>peanut</td>
</tr>
<tr>
<td>i1</td>
<td>honey bee</td>
</tr>
<tr>
<td>i3</td>
<td>wasp</td>
</tr>
</tbody>
</table>

Taking blood samples for Specific IgE testing after anaphylaxis

Specific IgE allergen tests can also be requested on blood samples taken after the anaphylactic event, and a raised allergen-specific IgE level may be useful. Due to the rather quick fall of drug-specific IgE levels, seen in some cases, it is best to test as soon as possible and not later than 6 months after the event. However, a negative result does not exclude IgE mediated anaphylaxis. An interval between the time of the allergic reaction and the appearance of measurable allergen-specific IgE antibodies has been observed in some cases. If an initial negative result is achieved it is therefore recommended to re-test with a new blood sample within 2-3 weeks after the anaphylaxis.

This is not an exhaustive list of allergens, please see the allergen database ImmunoCAP ‘Invitro sight’ on www.ImmunoCAP.com for full range of CE marked Specific IgE allergens and method details.
References:

1. Department of Health data.
2. Schwartz LB. Tryptase from human mast cells, biochemistry, biology and clinical utility. Monographs in Allergy 1990; 27: 90-113
5. Hepner DL, Castells M. Anaphylaxis during the perioperative period. Anaesth Analg. 2003;97: 1381-95
14. Personal Communication from Dr Richard Pumphrey, Consultant Immunologist St Mary’s Hospital, Manchester
15. Royal College of Pathologists Working party on Autopsy. January 2005

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