ImmunoCAP® Tryptase

Fluoroenzymeimmunoassay

Directions for Use 52-5210-EN/08

INTENDED USE

ImmunoCAP Tryptase is an *in vitro* test system for the quantitative measurement of tryptase in human serum or plasma (EDTA and Heparin). ImmunoCAP Tryptase assay is specifically for use with ImmunoCAP 100^{C}, a dedicated measuring instrument. It is intended for *in vitro* diagnostic use in conjunction with other clinical findings, and is to be used in clinical laboratories, as well as physician office laboratories.

SUMMARY AND EXPLANATION OF THE TEST

Tryptase is the most abundant protein in mast cells. ImmunoCAP Tryptase measures the total tryptase levels of all proforms of α-tryptase and ß-tryptase as well as mature ß-tryptase (1-3).

Human mast cells play a central role in inflammatory processes. They are activated particularly during allergic reactions releasing inflammatory mediators. The number of mast cells is increased in systemic mastocytosis and certain associated haematological abnormalities and malignancies (4-6).

Monomeric proforms of α-tryptase and/or ß-tryptase are spontaneously released into the circulation reflecting the number of mast cells and constitute the baseline level of tryptase in healthy individuals. Persistently elevated levels of tryptase proforms serve as a clinical marker of systemic mastocytosis, as well as mast cell leukemia and certain other mast cell associated haematological disorders. The monitoring of tryptase levels serves as a marker of therapy aimed to reduce the mast cell burden (5,7).

Mature ß-tryptase is a tetrameric neutral serine-protease stored in the secretory granules of the mast cells. Extracellular released mature ß-tryptase serves as a clinical marker of mast cell activation. Allergens are the most common triggering factors causing mast cell activation through an IgE-dependent mechanism, while the same outcome may be caused by other endogenous as well as exogenous stimuli through non-IgE-dependent mechanisms (8-10).

Mature ß-tryptase is transiently elevated in most cases of systemic anaphylactic reactions. The peak level is usually reached 15-120 minutes after onset of the reaction and tryptase levels then decline slowly within the next 3-6 hours. The return to baseline levels can generally be verified approximately 24 hours after the reaction (1,11).

Elevated basal serum tryptase and/or an underlying mastocytosis may be risk factors particularly in patients with history of severe reactions. This should for example be taken into consideration in venom immunotherapy (12-14).

Elevated tryptase levels in post-mortem samples may indicate a fatal anaphylactic reaction as a cause of death (15-16).

Elevated tryptase levels have been followed locally, e.g. in nasal fluid during allergic rhinitis (17).

PRINCIPLE OF THE PROCEDURE

Anti-tryptase, covalently coupled to ImmunoCAP, reacts with the tryptase in the patient 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 of the eluate is measured. To evaluate the test results, the response for the patient samples are transformed to concentrations with the use of a calibration curve.

REAGENTS

Reagents are packaged in separate units, each purchased separately. The two digit suffix (-01) on the article number may vary between countries.

All units are required to perform an assay, though, calibrators are not required for additional assays while the stored curve is valid.

The expiration date and storage temperature for each of the units are stated on the outer label. However, each component is stable until the date stated on each individual component's label.

Note! It is not recommended to pool any reagents.

ImmunoCAP Tryptase (Art No 10-9303-01)
(Fluoroenzymeimmunoassay for 48 determinations)

Tryptase Conjugate ß-Galactosidase-anti-tryptase (mouse monoclonal antibodies) Approximately 3.5 µg/ml Sodium azide 0.06 % Color coded blue; 2.8 ml	1 vial	Ready for use Store at 2-8 °C until expiration date **Do not freeze!**
Tryptase Curve Control 1 (CC-1) (human tryptase in buffer) Sodium azide 0.05 % Color coded yellow; 0.2 ml	4 single dose vials	Ready for use Store at 2-8 °C until expiration date
ImmunoCAP Anti-Tryptase (aTryp) (mouse monoclonal antibodies) Preservative* <0.003 %	3 carriers of 16 ImmunoCAP	Ready for use Store at 2-8 °C until expiration date

ImmunoCAP Tryptase Curve Control (Art No 10-9341-01)
(For 6 additional assay runs)

Tryptase Curve Control 1 (CC-1) (human tryptase in buffer) Sodium azide 0.05 % Color-coded yellow; 0.2 ml	6 single dose vials	Ready for use Store at 2-8 °C until expiration date

ImmunoCAP Tryptase Calibrators (Art No 10-9302-01)
(For one calibration curve)

Tryptase Calibrators (Cal-xx) (human tryptase in buffer) Conc. 1; 5; 12.5; 50 and 200 µg/l Sodium azide 0.05 % Color-coded yellow; 0.2 ml	5 single dose vials	Ready for use Store at 2-8 °C until expiration date

ImmunoCAP Development Solution (Art No 10-9478-01)
(Reagents for 600 determinations)

Development Solution 4-Methylumbelliferyl-ß-D-galactoside 0.01% Preservative* <0.0010%; 6.0 ml	6 vials	Ready for use Store at 2–8 °C until expiration date **Do not freeze!**

ImmunoCAP Stop Solution (Art No 10-9479-01)
(Reagents for 600 determinations)

Stop Solution Sodium carbonate 4 %; 65 ml	6 bottles	Ready for use Store at 2 - 8 °C until expiration date

ImmunoCAP Washing Solution (Art No 10-9422-01/10-9202-01)
For information see separate Washing Solution package insert.

***Preservative**: Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1).

⚠ Precautions

- For *in vitro* diagnostic use. Not for internal or external use in humans or animals.
- Do not use reagents beyond their expiration dates.
- This kit contains reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or other local/national guidelines on laboratory safety procedures.
- Reagents containing >0.0015% mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*isothiazol-3-one [EC no. 220-239-6] (3:1) may cause sensitisation by skin contact. Avoid contact with skin. Wear suitable gloves. For more information see Safety Data Sheet.
- Reagents that contain sodium azide as a preservative must be handled with care. For more information see Safety Data Sheet.

Handling of ImmunoCAP Carrier

Keep the carrier closed to avoid evaporation of buffer. Do not leave the carrier open for more than 1 day at room temperature, otherwise, discard the first ImmunoCAP.

Indication of instability

ImmunoCAP 100^{C} software has built-in acceptance limits for the calibration curve and the curve controls. For more information see ImmunoCAP 100^{C} User Manual.

INSTRUMENTS

ImmunoCAP 100^{C} with built-in software processes all steps of the assay and prints results automatically after the assay is completed. For further information regarding assay setup, instrumentation and software etc. see ImmunoCAP 100^{C} User Manual.

SPECIMEN COLLECTION AND PREPARATION

Serum and plasma (EDTA or heparin) samples from venous blood can be used. Collect blood samples and prepare serum or plasma according to standard procedures. Keep specimens at room temperature (RT) for shipping purposes only, up to 2 days. Store at 2 °C to 8 °C for up to one week, or else at –20 °C. Avoid repeated freezing and thawing.

It is preferable that samples be taken no earlier than 15 minutes from onset / up to 3 hours after the onset of the suspected incident causing mast cell activation (9,10). The time between the reaction and sample collection should be noted. To confirm the return to baseline levels an additional blood sample should be collected after 24 - 48 hours, time depending on the magnitude of the activation. At the suspicion of elevated basal levels or an underlying mastocytosis additional sample(s) should be taken 1-2 week(s) later.

Post mortem samples should be taken within 48 hours from time of death.

For information regarding the assay of tryptase in media other than human serum or plasma, please contact Phadia AB.

Preparation of Samples

No dilution of samples is usually required. However, for determination of values higher than 200 µg/l, samples can be diluted with:

ImmunoCAP IgE/ECP/Tryptase Sample Diluent (10-9256-01)

PROCEDURES

See ImmunoCAP 100^{C} User Manual for more information.

Parameters of the procedure
Volumes per determination:

Sample	40 µl
Conjugate	50 µl
Development Solution	50 µl
Stop Solution	600 µl

Total time for one assay is 2.5 hours.
Incubations are performed at 37 °C by ImmunoCAP 100^{c}.

Procedural steps
See ImmunoCAP 100^{c} User Manual for more information.

Material
Materials provided by Phadia AB:
See under REAGENTS.
Materials required but not provided by Phadia AB:
- Measuring cylinder 1000 ml
- Purified water

Additional products available from Phadia AB:

ImmunoCAP 100^{c} Instrument (12-3500-01)

ImmunoCAP Information Data Manager Software Package (12-3801-11)

ImmunoCAP Maintenance Solution Kit (10-9476-01)

ImmunoCAP FluoroC (10-9264-01)

Calibration
The calibrators (Tryptase Calibrators) are run in duplicates to obtain a full calibration curve. The curve can be stored. Use one Curve Control in duplicate to evaluate subsequent assays against the stored curve. For more information see ImmunoCAP 100^{c} User Manual.

Reference material
Tryptase calibrators are calibrated against an internal tryptase reference which has been purified from human lung according to Schwartz et al (18).

Measuring Range
The measuring range for an undiluted sample is 1-200 µg/l.

Quality control
Record keeping for each assay: It is good laboratory practice to record the lot numbers of the components used, the dates when they were first opened and the remaining volumes.
Control Specimens: Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptable ranges.
Controls available from Phadia AB for day to day quality control:

ImmunoCAP Tryptase Control (10-9370-01)

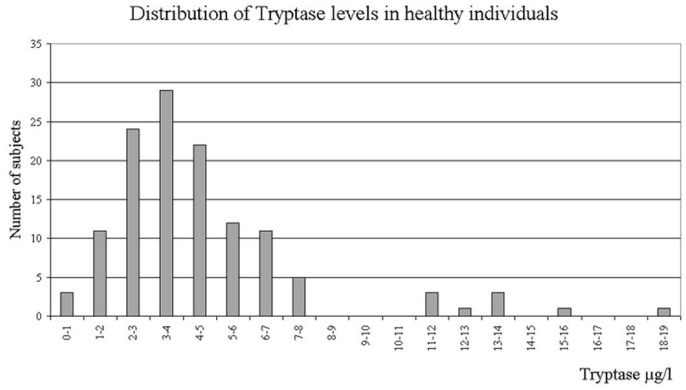
CALCULATION OF RESULTS
ImmunoCAP 100^{c} is programmed to automatically calculate all results. For more information, see ImmunoCAP 100^{c} User Manual.

LIMITATIONS OF THE PROCEDURE
A definitive clinical diagnosis should not be based on the results of any single diagnostic method and should only be made by the physician after all clinical and laboratory findings have been evaluated.
- Systemic anaphylactic reactions without elevation of tryptase might be caused by other non-mast cell pathways.
- In general, elevated tryptase levels are more commonly seen after parenteral than oral introduction of agents causing systemic anaphylactic reactions.
- Moderately elevated tryptase levels have been observed in post-mortem samples by other death causes than anaphylactic reactions.
- Heterophilic antibodies, especially human-anti mouse antibodies (HAMA), in human serum/plasma might react with the mouse immunoglobulins used as capturing antibodies on the solid phase and as detection antibodies in the conjugate in ImmunoCAP Tryptase (19, 20). The presence of heterophilic antibodies is uncommon but can cause false results, mostly falsely elevated levels. The composition of the anti-tryptase conjugate is designed in order to minimize this kind of interference.
  Still uncommon, the risk for interference is increased in certain patient groups e.g. patients having rheumatoid factor (RF) or patients receiving preparations containing mouse monoclonal antibodies (including chimeric/humanized) for diagnostic and/or therapeutic use. Another risk group is patients regularly exposed to animals and/or animal products.
  One method to eliminate heterophilic antibodies is by pre-treatment of samples using commercially available Heterophilic Blocking Tubes (HBT).

EXPECTED VALUES[(a)]
Good laboratory practice recommends that each laboratory establishes its own expected range of values.
A study with 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:

Geometric mean	3.8 µg/l
95 upper percentile	11.4 µg/l

The distribution is shown in figure.



In patients with systemic mastocytosis levels of tryptase are, in general, persistently elevated above 20 µg/l (5).
Baseline tryptase levels in the range of approximately 10-20 µg/l reflect an increased mast cell burden indicating an increased risk in patients with history of severe anaphylactic reaction (1).
In severe cases the triggering agent causing a transiently elevation of tryptase should be identified.

PERFORMANCE CHARACTERISTICS
Precision[(a)]
The following mean coefficients of variation have been calculated. Each sample has been assayed in 4 replicates on 27 different occasions. The study included 3 lots of conjugate, 2 lots of calibrators and 2 lots of ImmunoCAP. A total of 5 calibration curves was used for evaluation.

Serum level (µg/l)	Coefficients of variation (%)	
	Within assay	Between assay
3.8	3.4	4.5
12	2.9	4.4
36	2.6	3.7
108	2.2	2.1

Sensitivity[(a)]
The detection limit is <1.0 µg/l (3 SD from zero standard).

Specificity[(a)]
Cross-reactivity was tested with the following result:
Heparin <0.01%

Recovery[(a)]
Known amounts of human tryptase were added to human serum samples.
Mean recovery is 97 ± 8%

WARRANTY
The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Phadia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

SYMBOLS USED

LOT	Batch Code	IVD	*In Vitro* Diagnostic Medical Device
	Biological Risks		Caution
	Temperature Limitation		Contains Sufficient for <n> Tests
	Use By		Consult Instructions for Use
	Manufacturer		

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ImmunoCAP is our brand name and replaces the old name UniCAP.

Notes

[a]Studies performed at Phadia AB, Uppsala, Sweden.

Patents/Trademarks

Brand name change

Phadia AB is changing the brand names of the instrument platforms from "ImmunoCAP®" to "Phadia®". The new name is being applied to the instruments and related items e.g. Software and User Manuals. The System Reagents will have the "ImmunoCAP®" brand name removed. This is a brand name change only; the change has no impact on performance or safety. "Phadia®" and "ImmunoCAP®" may be used interchangeably in this Directions for Use / User Manual and in other related labeling.

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