ImmunoCAP™ Tryptase

Fluoroenzymeimmunoassay

Rx only

CLIA Complexity Category = Moderately Complex

Directions for Use 52-5679-US/03

INTENDED USE

ImmunoCAP Tryptase is an in vitro semi-quantitative assay for measurement of tryptase in human serum or plasma (EDTA, lithium heparin or sodium heparin). It is intended for in vitro diagnostic use as an aid in the clinical diagnosis of patients with a suspicion of systemic mastocytosis in conjunction with other clinical and laboratory findings. ImmunoCAP Tryptase is to be used with the instrument Phadia 100.

SUMMARY AND EXPLANATION OF THE TEST

Human mast cells play a central role in inflammatory processes. During IgE mediated allergic reactions they are activated and release inflammatory mediators including tryptase (1, 2, 3).

Tryptase is the most abundant protein in mast cells. ImmunoCAP Tryptase measures the total tryptase levels including all forms of α-tryptase and ß-tryptase.

The baseline level of tryptase in the circulation reflects the number of mast cells. Elevated baseline levels of tryptase are an indication of mastocytosis and measurement of tryptase is recognized by the WHO as a minor diagnostic criterion of this disease (4, 5, 6, 7).

PRINCIPLE OF THE PROCEDURE

Anti-tryptase, covalently coupled to ImmunoCAP, reacts with the tryptase in the patient sample. After washing, enzyme labeled antibodies against tryptase are added to form a complex. Following 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. The higher the response value, the more tryptase is present in the sample. To evaluate the test results, the responses for the patient samples are transformed to concentrations with the use of a calibration curve.

REAGENTS AND MATERIAL

Reagents are packaged as described below, each purchased separately.

The expiration date and storage temperature are stated on the labels. Do not use reagents beyond their expiration dates.

Note: It is not recommended to pool any reagents.

Keep the ImmunoCAP carrier closed to avoid evaporation of buffer.

Reagents for Phadia 100

- **ImmunoCAP Tryptase** (Art No 10-9525-02: for 48 determinations)
  - Tryptase Conjugate (1 vial)
  - Tryptase Curve Control 1 (CC-1) (4 single dose vials)
- **ImmunoCAP Tryptase Calibrators** (Cal-xx) (Art No 10-9526-01: for 1 calibration curve)
- **ImmunoCAP Tryptase Curve Control** (CC-1) (Art No 10-9527-01: for 6 additional assay runs)
- **ImmunoCAP Tryptase Anti-Tryptase** (a-Tryp) (Art No 14-4518-01: carrier of 16 ImmunoCAP)
- **Development Solution** (Art No 10-9478-01: for 600 determinations)
- **Stop Solution** (Art No 10-9479-01: for 600 determinations)
- **Washing Solution** (Art No 10-9422-01: 6 x 1 l)
  - Washing Solution Additive, 6 x 17.2 ml
  - Washing Solution Concentrate, 6 x 80 ml
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Tryptase Control** (Art No 10-9370-01: 6 vials)

Details of reagents

ImmunoCAP Tryptase Conjugate	
ß-Galactosidase-anti-tryptase Approximately 1.6 µg/ml (mouse monoclonal antibodies) Sodium azide 0.06%	Ready for use. Store at 2 – 8 °C until expiration date. **Do not freeze!**

ImmunoCAP Tryptase Calibrators	
(human recombinant tryptase in buffer) Conc. 1; 5; 12.5; 50 and 200 µg/l Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Tryptase Curve Control	
(human recombinant tryptase in buffer) Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Tryptase Anti-Tryptase	
(mouse monoclonal antibodies) Preservative* <0.0015%	Ready for use. Store at 2 – 8 °C until expiration date.

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

Stop Solution	
Sodium carbonate 4%	Ready for use. Store at 2 – 32 °C until expiration date.

Washing Solution
For information, see separate Directions for Use for Washing Solution.

ImmunoCAP Tryptase Control
ImmunoCAP Tryptase Control is prepared from selected pooled human sera. It is lyophilized to ensure maximum stability. Approximately target value 15 µg/l. **Note:** Please refer to the vial label for specific assayed target range. **Reconstitution of ImmunoCAP Tryptase Control** Reconstitute the content of a vial by adding exactly 500 µl purified water (8, 9) or clinical laboratory reagent water (CLRW, 10). Let the vial stand for one minute, then mix gently until the content is completely dissolved. **Shelf-life and storage** Lyophilized serum: Store at 2-8 °C until expiration date. Reconstituted serum: Store at 2 – 8 °C for 1 week or -20 ° C for 4 weeks. (Repeated freezing and thawing should be avoided.)

ImmunoCAP IgE/ECP/Tryptase Sample Diluent	
(buffer solution with Bovine Serum Albumin) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

***Preservative:** Reaction mass of CMIT/MIT (3:1), (CAS No: 55965-84-9).

Additional material

Additional products available from Phadia AB:

- ImmunoCAP IgE/ECP/Tryptase Sample Diluent (10-9256-01/10-9360-01)

Materials required but not provided by Phadia AB:

- Measuring cylinder 1000 ml
- Purified water (8, 9) or Clinical Laboratory Reagent Water (CLRW, 10)

Precautions

- For in vitro diagnostic use. Not for internal or external use in humans or animals.
- Some reagents are 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% reaction mass of CMIT/MIT (3:1) (CAS No: 55965-84-9) may cause an allergic skin reaction (H317). Wear protective gloves/protective clothing/eye protection (P280). Gloves: Nitrile rubber EN374. For more information see Safety Data Sheet.
- Reagents that contain sodium azide as a preservative must be handled with care. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with large volume of water to prevent azide build-up. For more information refer to the Safety Data Sheet and other local/national guidelines.

INSTRUMENTS

Phadia 100 processes all steps of the assay and prints results automatically after the assay is completed[(a)].

Phadia 100 has no provisions for on board reagent storage.

SPECIMEN COLLECTION AND PREPARATION

Specimen collection

Serum and plasma (EDTA, lithium heparin or sodium 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 (18 °C to 32 °C) 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 (11).

Note: It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory. In general, laboratories should perform validation studies before implementing a change in specimen acceptance criteria (11).

Preparation of samples

Sample dilution is usually not required.

For determination of values higher than 200 µg/l, dilute the samples with ImmunoCAP IgE/ECP/Tryptase Sample Diluent.

Handling of control specimen

It is recommended to remove and recap the control vials from the instrument as soon as the pipetting of the samples is finished and the sample incubation is started. It is also recommended to gently stir the vial before use. Avoid repeated freezing and thawing.

ImmunoCAP Tryptase Control should be treated in the same way as a patient sample in the procedure.

PROCEDURES

Procedural steps[(a)]

For procedural steps, see **Notes** a.

Parameters of the procedure

Patient samples are run in single determinations.

Volumes per determination:

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

Incubations are performed at 37 °C by Phadia 100 instrument.

Total time for one assay is 2.5 hours.

Calibration[(a)]

The ImmunoCAP Tryptase Calibrators are run in duplicates to obtain a full calibration curve. The curve can be stored. The software for Phadia instruments have built-in acceptance limits for the calibration curve and the curve controls. Use one curve control, CC-1, in duplicate determinations to evaluate subsequent assays against the stored curve.

Calibrator range: 1-200 µg/l.

Reference material: ImmunoCAP Tryptase Calibrators are calibrated against an in-house tryptase reference which traces back to tryptase purified according to Schwartz et al (12).

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 specimen

Good laboratory practice requires that quality control specimen 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)

Intended use

ImmunoCAP Tryptase Control is used for monitoring ImmunoCAP Tryptase measurements performance in Phadia instruments.

Expected values for ImmunoCAP Tryptase Control

As with all immunoassays the results are affected by the testing procedures and equipment used by different laboratories. It is therefore recommended that each laboratory establishes

its own target value for each actual lot of control together with criteria of acceptance (recommended range ± 30 %).

This established target value is expected to fall within the range for the actual lot stated on the vial label.

The range for each specific lot is calculated as a mean ± 3 SD using the expected long term variation. The mean value for each specific lot has been determined from 6 consecutive control assays, each in 6 replicates using ImmunoCAP Tryptase.

RESULTS

Phadia 100 is programmed to automatically calculate all results[(a)]. For Phadia 100 using Phadia Information Data Manager Software, all calculations are automatically performed[(a)].

LIMITATIONS OF THE PROCEDURE

- Not for standalone diagnosis of systemic mastocytosis.
- Not for diagnosis of anaphylaxis, or for evaluation of a potential anaphylactic reaction.
- A definitive clinical diagnosis should only be made by the physician after all clinical and laboratory findings have been evaluated. It should not be based on the results of any single diagnostic method.
- 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 (13, 14). 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 confirm the presence of heterophilic antibodies is by pre-treatment of samples using commercially available Heterophilic Blocking Tubes (HBT). For use and limitations of HBT, see manufacturer's instructions.

EXPECTED VALUES[(b)]

Good laboratory practice recommends that each laboratory establishes its own expected range of values.

Studies on two sites were performed to evaluate the clinical performance of ImmunoCAP Tryptase on in total 138 patients with a suspicion of systemic mastocytosis. Site 1 included 54 patients aged 2-75 years, 21 of them with a positive diagnosis of systemic mastocytosis according to WHO criteria. Site 2 included 84 patients aged 5-76 years, 56 of them with a positive diagnosis of systemic mastocytosis according to WHO criteria.

Using the cut-off 20 µg/l, the clinical sensitivity and specificity was:

Study	Clinical sensitivity	Clinical specificity
Site 1	76%	67%
Site 2	66%	86%

A study(b) with 163 self-reported healthy individuals (70 males and 93 females) was performed. The age range was 0-68 years for both males and females.

The following ImmunoCAP Tryptase results were obtained:

Geometric mean	3.6 µg/l
95 upper percentile	8.2 µg/l

In patients with systemic mastocytosis levels of tryptase are, in general, persistently elevated above 20 µg/l (7).

PERFORMANCE CHARACTERISTICS

Precision[(b)]

The following pooled coefficients of variation have been obtained with Phadia 100 and 4-10 samples/level. Each sample has been assayed in 4 replicates on 7 different occasions on each of 3 different instruments, using the same lot of reagents and stored calibration curves.

Sample level (µg/l)	Coefficients of variation (%)	
	Within assay	Between assay
1-20	3	3
20-100	3	3
100-200	3	4

Analytical sensitivity[(b)]

The limit of detection (15) is <1.0 µg/l.

Analytical specificity[(b)]

No interference of Heparin, Rheumatoid factor, hemolysed, lipemic or icteric samples has been observed.

Recovery[(b)]

Mean recovery is 93.3% (SD 4.5%).

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

	Use-by date	Σ	Contains sufficient for <*n*> tests
LOT	Batch code	IVD	*In vitro* diagnostic medical device
	Date of manufacture		Temperature limit
REF	Catalogue number		Consult instructions for use
	Caution		Biological risks
	Manufacturer	**Rx only**	Prescription use only

Full symbol glossary is available at: https://symbols_glossary.phadia.com.

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Notes

[(a)]For more information, see Phadia 100 User Manual.

[(b)]Studies performed at Phadia AB, Uppsala, Sweden.

Patents/Trademarks

Addresses

USA Phadia US Inc.
4169 Commercial Avenue
Portage, Michigan 49002
Tel: +1 800-346-4364 (Toll Free) Fax: +1 269 492-7541

Phadia AB,
Rapsgatan 7P, P. O. Box 6460, 751 37 Uppsala, Sweden
Tel: +46 18 16 50 00 Fax: +46 18 14 03 58

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