**SUMMARY AND EXPLANATION OF THE TEST**

ImmunoCAP™ Tryptase

Details of reagents

- **ImmunoCAP Tryptase Control**
  - Washing Solution
  - Stop Solution

- **ImmunoCAP Tryptase Curve Control**
  - (human recombinant tryptase in buffer)

- **ImmunoCAP Tryptase Anti-Tryptase**
  - (mouse monoclonal antibodies)

- **Development Solution**
  - 4-Methylumbelliferyl-ß-D-galactoside

- **Stop Solution**
  - Sodium carbonate 4%

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

**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 date.

- For more information refer to the Safety Data Sheet and other local/national guidelines.

**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 treated for immunization against 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. (DOH) 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.

**INSTRUMENTS**

Phadia 100 processes all steps of the assay and prints results automatically after the assay is completed(26). 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 up to one week, or else at -20 °C. Avoid repeated freezing and thawing (11).

**Handling of control specimen**

It is recommended to remove and recapt 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**

For procedural steps, see Notes a.

**Parameters of the procedure**

- **Patient samples are run in single determinations.**
  - Volumes per determination:
    - Sample: 40 µl
    - Controls: 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**

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. Calibration 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 test 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.

**INSURANCE**

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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(b).

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-trypsin 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 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
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 2-76 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:

<table>
<thead>
<tr>
<th>Study</th>
<th>Clinical sensitivity</th>
<th>Clinical specificity</th>
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</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>76%</td>
<td>67%</td>
</tr>
<tr>
<td>Site 2</td>
<td>66%</td>
<td>86%</td>
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</tbody>
</table>

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**
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.

<table>
<thead>
<tr>
<th>Sample level (μg/l)</th>
<th>Coefficients of variation (%)</th>
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<tbody>
<tr>
<td></td>
<td>Within assay</td>
</tr>
<tr>
<td>1-20</td>
<td>3</td>
</tr>
<tr>
<td>20-100</td>
<td>3</td>
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</tbody>
</table>

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

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

**Recovery**
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
- Batch code
- Date of manufacture
- Catalogue number
- Reference
- Caution
- Manufacturer
- Rx

**REFERENCES**