1. Introduction

In humans, histamine (b-imidazole-ethylamine) is the most important mediator and is mostly found in the initial phase of an anaphylactic reaction ("immediate type" allergy). Histamine is developed by the enzymatic decarboxylation of histidine. In the organism, histamine is present in nearly all tissues, and it is mainly stored in the metachromatic granula of mast cells and the basophilic leukocytes. It is present in an inactive bound form and is only released as required.

Histamine acts predominately on smooth muscle and blood vessels. In humans, it is responsible for the bronchoconstriction occurring during the acute phase. In the vessels, its constrictive effect is limited to the venula, where arterioles are dilated. Furthermore, histamine causes a contraction of the cells of the vascular endothelium and increases the vascular permeability, thereby allowing higher-molecular substances to escape into the tissue.

Like several other mediators, histamine does not only mediate various clinical symptoms of anaphylaxis but also induces a series of effects which are directed towards a termination of the anaphylactic reaction. Histamine may inhibit the release of lysosomal enzymes from polymorphonuclear leukocytes, the degranulation of mast cells and basophils, and the production of complement components through mononuclear phagocytes. Furthermore, histamine can activate suppressor T cells and, thus, may inhibit the production of IgE. The biological action of histamine in tissue is mediated by three different receptors, i.e. H1, H2, and H3 receptors.

Of clinical interest in the histamine determination is the quantification of the histamine release from basophilic leukocytes in allergies of the "immediate type" as well as of the histamine quantity which is present in various body fluids (plasma, urine, cell culture supernatants), after allergen administration. First contact of the organism with an allergen does not result in initiation of a histamine release. First, specific IgE antibodies are produced which migrate to the mast cells and they bind to the receptors. At the second allergen contact, a transformation of a B cell to a plasma cell is no longer required. The allergen directly moves to
the IgE antibodies already bound to the mast cells and binds to these antibodies. The mast cell responds by histamine secretion from its granula.

2. Principle of the Test

Heparinized whole blood samples are incubated with different concentrations of the suspected allergen. Release of histamine will occur upon stimulation of basophilic granulocytes depending on their sensibility to the allergen. The released histamine in the supernatant is subsequently determined using a specific plasma immunoassay, the Histamine ELISA or RIA purchased in connection with this kit.

This histamine value is related to the 100% control (= Total Histamine) and the blank value (= Spontaneous Release).

The determination of the in vitro release of histamine represents a sensitive and specific method as well as a suitable addition to routine diagnostic procedures including conventional skin testing and radioallergosorbent tests (RAST) for the determination of specific IgE antibodies in serum of atopic patients. In addition, this test also detects the “releasability” of the cells.

The direct detection of mediator substances like histamine during an allergic reaction is not only of scientific interest but also of practical significance in connection with a specific antagonistic therapy.

This test is also very suitable for the analysis of pathophysiological responses to drugs, chemicals, and other compounds which have not been evaluated for adverse side effects.

3. References


4. Precautions

– The assay reagents contain sodium azide or thimerosal which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
– This kit is for in vitro diagnostic use only.
– Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
– Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
– Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

5. Storage and Stability

The kit is shipped at ambient temperature and should be stored in the dark at 2-8°C. Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

6. Reagents Supplied

6.1 Release Buffer

1 bottle
Lyophilized.
dissolve in 20 ml distilled water.

6.2 Anti-IgE
1 vial
Lyophilized, dissolve in 2 ml Release Buffer.

**6.3 Hypotonic Medium**

1 bottle
20 ml, ready to use.

**Materials Required but not Supplied:**

– Pipettes
– Vortex mixer
– Glass test tubes (12 x 75 mm)
– Temperature controlled water bath, 37°C
– Centrifuge

**7. Preparation of Reagents**

**7.1 Release Buffer**

Pipette 20 ml of distilled water to the lyophilized buffer preparation. Leave for 15 minutes before swirling gently.

The dissolved buffer is stable for 3 days at 2-8°C. For longer storage, store at -20°C or below.

**7.2 Anti-IgE**

Pipette 2 ml of the ready to use Release Buffer to the lyophilized antibody preparation. Leave for 15 minutes before swirling gently.

The dissolved buffer is stable for 3 days at 2-8°C. For longer storage, store at -20°C or below.

**8. Specimen Collection and Storage**

The histamine release is performed with heparinized whole blood. The samples should be mixed carefully immediately after collection.

24 h before drawing blood samples, the patient should avoid taking any allergy causing drugs, antihistaminics, oral corticosteroids, and substances which block H₂ receptors.

The histamine release should be performed within 24 hours after sample collection. The blood samples should be stored at room temperature (at 2-8°C the leukocytes would clot).

**8.1 Calculation of sample volumes for Histamine ELISA:**

50 µl for total histamine content
200 µl for spontaneous release
200 µl for positive control with anti IgE
200 µl for each allergen concentration

Example: For a test with 2 allergens and 5 concentrations each, you will need 2.45 ml of blood.

**8.2 Calculation of sample volumes for Histamine RIA:**

50 µl for total histamine content
400 µl for spontaneous release
400 µl for positive control with anti IgE
400 µl for each allergen concentration

Example: For a test with 2 allergens and 5 concentrations each, you will need 4.85 ml of blood.

**9. Preparation of the Allergen**

Prepare several 10-fold dilutions (e.g. from 10⁻¹ to 10⁻⁵) using a stock allergen solution of 1 mg/ml.

**9.1 Histamine ELISA**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Allergen Extract</th>
<th>Release Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻¹</td>
<td>30 µl stock allergen solution (1 mg/ml)</td>
<td>270 µl</td>
</tr>
<tr>
<td>10⁻²</td>
<td>30 µl of 10⁻¹ dilution</td>
<td>270 µl</td>
</tr>
</tbody>
</table>
9.2 Histamine RIA

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Allergen Extract</th>
<th>Release Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>60 µl stock allergen solution (1 mg/ml)</td>
<td>540 µl</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>60 µl of $10^{-1}$ dilution</td>
<td>540 µl</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>60 µl of $10^{-2}$ dilution</td>
<td>540 µl</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>60 µl of $10^{-3}$ dilution</td>
<td>540 µl</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>60 µl of $10^{-4}$ dilution</td>
<td>540 µl</td>
</tr>
</tbody>
</table>

10. Test Procedure

10.1.1 Histamine ELISA

– Label glass tubes according to the following scheme for each allergen and each patient. Swirl whole blood samples gently before pipetting.

Anti-IgE Allergen Dilution Whole Blood Release Buffer
Tube(µl)(µl)(µl)(µl)
$10^{-1}$-200200-
$10^{-2}$-200200-
$10^{-3}$-200200-
$10^{-4}$-200200-
$10^{-5}$-200200-
etc..-(200)(200)-
Spontaneous--200200
Positive Control200-200-

– Mix the tubes carefully, avoiding drops at the tube wall.
– Incubate in a water bath for 60 minutes at 37°C.
– Stop the release by incubating for 10 minutes in an ice bath.
– Centrifuge for 10 minutes at 700 x g*.
– Withdraw 100 µl of the clear supernatant for the Histamine ELISA (avoid taking cells from the pellet).

* g (relative centrifugal force) is not equivalent to rounds per minute (rpm). The rpm value has to be calculated depending on the radius (r) of the centrifuge.

10.1.2 Histamine RIA
Label glass tubes according to the following scheme for each allergen and each patient. Swirl whole blood samples gently before pipetting.

**Anti-IgE Allergen Dilution**

<table>
<thead>
<tr>
<th>Whole Blood</th>
<th>Release Buffer</th>
<th>Tube(µl)</th>
<th>(µl)</th>
<th>(µl)</th>
<th>(µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10(^{-1})</td>
<td>400</td>
<td>400</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10(^{-2})</td>
<td>400</td>
<td>400</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10(^{-3})</td>
<td>400</td>
<td>400</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10(^{-4})</td>
<td>400</td>
<td>400</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>etc.</td>
<td>(400)</td>
<td>(400)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Spontaneous Release

Positive Control 400-400

Mix the tubes carefully, avoiding drops at the tube wall.

– Incubate in a water bath for **60 minutes at 37°C**.

– Stop the release by incubating for **10 minutes in an ice bath**.

– Centrifuge for **10 minutes at 700 \( \times \) g**.

– Withdraw **2 x 100 µl** of the clear supernatant for the Histamine RIA (avoid taking cells from the pellet).

**10.2 Total Histamine (ELISA and RIA)**

In order to release the total histamine from the leukocytes:

– Add **950 µl** of **Hypotonic Medium** to 50 µl of heparinized whole blood (use glass tubes; 1/20 dilution).

– Incubate for **60 minutes at 37°C** in a waterbath.

– Mix on a Vortex mixer.

– Withdraw **100 µl** for the Histamine ELISA, or **2 x 100 µl** for the Histamine RIA.

**10.3 Storage of Samples**

The supernatants of the respective samples can be stored at 2-8°C for one day. For longer storage, freeze at -20°C. Avoid repeated thawing and freezing.

**11 Immunoassay**

In order to detect any influences of the allergen preparation, one or more of the dilutions should be run as additional allergen control in the assay.

**11.1 Histamine ELISA**

The acylation required is performed according to the following scheme:

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Release</th>
<th>Allergen</th>
<th>Kit</th>
<th>Standards</th>
<th>Supernatant</th>
<th>Control</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td>50 µl</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Release</td>
<td>Supernatant</td>
<td>100 µl</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergen</td>
<td>Control</td>
<td>50 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kit Controls</td>
<td>---</td>
<td>50 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Release Buffer</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicator Buffer</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vortex Mix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acylation Reagent</td>
<td>20 µl</td>
<td>20 µl</td>
<td>20 µl</td>
<td>20 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vortex Mix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubate for 30 minutes at room temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>0.75 ml</td>
<td>0.75 ml</td>
<td>0.75 ml</td>
<td>0.75 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vortex Mix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Afterwards, follow Section 10 (Test Procedure) of ELISA instructions.

**11.2 Histamine RIA**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Release</th>
<th>Allergen</th>
<th>Kit</th>
<th>Standards</th>
<th>Supernatant</th>
<th>Control</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>150 µl</td>
<td>50 µl-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The table and the scheme for acylation and incubation are detailed instructions for the histoamine assays, including the use of standards, controls, and supernatants. The procedures involve careful mixing and incubation to ensure accurate results.
Afterwards, follow the test procedure in the RIA instructions.

12. Calculations of Results

Due to the dilution factor of 1:2, the histamine concentration of the unknown samples (spontaneous release, positive control, release supernatant, allergen control) can be read directly from the standard curve.

For the total histamine the value has to be corrected by multiplying with 10 (due to the 20-fold dilution during histamine release).

The result of the spontaneous release has to be subtracted from the amount of the unknown samples and the total histamine. Calculate the percentage of each sample, regarding the total histamine content as 100%. Plot a curve of the percentage release against the allergen concentration on semi-logarithmic paper.

The degree of cellular sensitization (HR_{50}) is defined as the allergen concentration which leads to 50% histamine release in relation to the total histamine. If the release is lower than 50%, the HR_{30} (allergen concentration which is capable of releasing 30% of the total histamine) can be used.

13. Interpretation of Results

Total histamine in whole blood: 20-200 ng/ml.

Spontaneous Release: This value should be < 5% of the total histamine content. Higher values indicate cell damage.

Positive Control: This value should be > 5% of the total histamine content.

Allergen Induced Release: Any positive signal greater than 5% release (after subtraction of the spontaneous release value) has to be regarded as allergen specific.

14. Warranty

Any modification of this test as well as exchange or mixture of any components from different lots might influence the results. In such cases there is no claim for a replacement.