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Instructions for use

Histamine Release

REF **IB89145R**

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Histamine Release (Supplementary kit)

1. Introduction

1.1 Intended use and principle of the test

Supplementary kit for the quantitative determination of the release of histamine from heparinized whole blood (this kit has to be used in combination with the Histamine ELISA, for details contact your local supplier).

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

Histamine acts predominantly on smooth muscle and blood vessels. In humans, it is responsible for the broncho-constriction occurring during the acute phase. In the vessels, its constrictive effect is limited to the venula, whereas 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 exclusively 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 basophiles 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 guaranteed by three different surface receptors, i.e. H1, H2 and H3 receptors.
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 purchased in connection with this kit. This histamine value is related to the 100% control (= Total Histamine) and the blank value (= Spontaneous Release).

1.2 Background

Please refer to the instructions for use of the Histamine ELISA, **chapter 1.2**.

2. Procedural cautions, guidelines, warnings and limitations

Please refer to the instructions for use of the Histamine ELISA, **chapter 2**.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA E-1145	ANTI-IGE	**Anti-IgE-Antiserum -** Concentrated
Contents:	Rabbit Anti-Human IgE antibody	
Volume:	1 x 25 µl/tube	
BA E-1126	RELEASE-BUFF	**Release Buffer** – Ready to Use
Contents:	Buffer with physiological pH	
Volume:	1 x 50 ml/vial, orange cap	

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 5 – 500 µl
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Centrifuge capable of at least 3.000 x g
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Temperature controlled water bath (37 °C and 90 °C) or similar heating device
- Histamine ELISA, Vortex mixer, water (deionized, distilled, or ultra-plus)

5. Sample collection and storage

Heparinized Whole Blood

24 h before drawing blood samples the patient should not ingest any allergy causing drugs, antihistaminics, oral corticosteroids and substances which block H2 receptors.

The Histamine Release is performed with heparinized whole blood. That means whole blood is collected into a tube (e.g. MonovetteTM or VacuetteTM) according to the manufacturer's instructions that contains heparin as anti-coagulant.

The samples can be stored for up to 24 hours at room temperature. Please do not keep the samples refrigerated, this will lead to clotting of the leucocytes. Avoid direct sunlight.

The following quantities of whole blood are needed:

- 40 µl for the determination of total histamine
- 200 µl for the spontaneous release
- 200 µl for positive control with anti- IgE
- 200 µl for each individual allergen concentration

Example: for a histamine release determination with 4 allergens in 3 different concentrations one would need approx. 2.5 ml of heparinized blood.

Storage:	20 - 25 °C	Keep away from heat or direct sun light.
Stability:	24 h	Avoid cooling. At 2 - 8 °C, the leucocytes will clot.

Storage of Supernatants: see chapter 6.4

6. Test procedure

6.1 Preparation of reagents

Anti-IgE-Antiserum Concentrate:

Prepare the working solution of the Anti-IgE-Antiserum by adding 5 µl of the Anti-IgE-Antiserum Concentrate to 5 ml of Release Buffer.

Storage of diluted Anti-IgE-Antiserum:	2 – 8 °C	- 20 °C
Stability of diluted Anti-IgE-Antiserum:	3 d	1 month

6.2 Release of the allergen induced histamine

Allergen dilutions

Starting with a stock solution of 1 mg allergen per ml water (deionized, distilled, or ultra-pure), a set of 10x dilutions is prepared according to the following pipetting scheme (example for 3 dilution steps):

No.	Dilution	Allergene solution	Release-Buffer
1	10^{-1}	50 µl stock- solution	450 µl
2	10^{-2}	50 µl solution No. 1	450 µl
3	10^{-3}	50 µl solution No. 2	450 µl

6.3 Histamine Release protocol (example for 3 dilution steps)

For each allergen tested with a patient the following pipetting scheme has to be followed:

Dilution	Allergen - dilution	Heparinized whole blood	Release Buffer	Anti-IgE Antiserum
10^{-1}	200 µl	200 µl		
10^{-2}	200 µl	200 µl		
10^{-3}	200 µl	200 µl		
Spontaneous - Release		200 µl	200 µl	
Positive - Control		200 µl		200 µl

6.4 Pipetting scheme Release

	allergen induced release	positive control	spontaneous release	total Histamine
Release-Buffer			200 µl	360 µl
allergene dilution	200 µl			
anti-IgE		200 µl		
Heparinized Whole Blood	200 µl	200 µl	200 µl	40 µl
mix carefully incubate for 60 min at 37 °C				mix carefully incubate for 10 min at 90 °C
Incubate for 10 min in an ice bath Centrifuge for 10 min at 700 x g (brake switched-off) Take 50 µl for the Acylation				

Storage of Supernatants

2 – 8 °C	-20 °C
1 d	1 week

6.5 Acylation

The Acylation has to be done with the Histamine ELISA test kit, starting with Chapter 6 Test procedure of the test instructions.

6.6 Histamine ELISA

The quantification of the Histamine has to be done with the Histamine ELISA test kit.

7. Calculation of results

Measuring range	Histamine
	0.12 - 50 ng/ml
	108 – 450 nmol/ml

The histamine concentrations of the samples from the **release test** can be read directly from the standard curve of the Histamine ELISA.

⚠ *The Histamine ELISA is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The results for the **total histamine** have to **be multiplied by factor 5**.

The histamine concentration from spontaneous release has to be subtracted from the allergen-induced histamine.

Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

Total Histamine	< 60 ng/ml
Spontaneous Release	< 5 % of total histamine value
Positive Control	> 5 % of total histamine value
Allergen Induced Release	> 5 % of total histamine value

7.1 Quality control

The valid confidence limits for the kit controls are listed in the QC-Report included in the kit.

8. Assay characteristics

Please refer to the instructions for use of the Histamine ELISA, **chapter 8**.

9. References/Literature

Please refer to the instructions for use of the Histamine ELISA, chapter 9.

⚠ ***For updated literature or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!