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Labor Diagnostika Nord GmbH & Co. KG

Instructions for use

Histamine Release

REF **BA 10-1100**

96

+2 +8 °C

IVD

CE

Histamine Release (Supplementary kit)

1. Intended use and principle of the test

Supplementary kit for the quantitative determination of Histamine Release in heparinized whole blood.

This kit has to be used in combination with the Histamine ELISA (BA E-1000)

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.

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 the initiation of a histamine release. Initially, specific IgE antibodies are produced which migrate to the mast cells and there 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 granules.

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

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 (REF BA E-1000) 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 anbodies 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.

2. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

3. Contents of the kit

BA E-1126	RELEASE-BUFF	**Release Buffer**	1 x 50 mL	ready for use
BA E-1145	ANTI-IGE	**Anti-IgE-Antiserum Concentrate**	1 x 10 µL	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.

4. Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL /100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Centrifuge capable of at least 3.000 x g
- Plate shaker (shaking amplitude 3mm; approx. 600 rpm), Absorbent material (paper towel)
- Vortex mixer, Histamine ELISA (Cat. No.: BA 10-1000)
- Temperature controlled water bath (37 °C and 90°C) or similar heating device, Distilled water

5. Sample collection and storage

Heparinized Whole Blood

24 h before drawing the 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. The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed.
The samples should be mixed carefully immediately after collection.

The following quantities of whole blood are needed:

- 30 µL for the determination of total histamine
- 150 µL for the spontaneous release
- 150 µL for positive control with anti- IgE
- 150 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:

2 – 8 °C	- 20 °C
1 d	1 week

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 mon

6.2 Release of the allergen induced histamine

Allergen dilutions
Starting with a stock solution of 1 mg allergen per mL dest. water, 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}	150 µL	150 µL		
10^{-2}	150 µL	150 µL		
10^{-3}	150 µL	150 µL		
Spontaneous - Release		150 µL	150 µL	
Positive - Control		150 µL		150 µL

6.4 Pipetting scheme Release

	allergen induced releases	positive control	spontaneous release	total Histamine
Release-Buffer			150 µL	270 µL
allergene dilution	150 µL			
anti-IgE		150 µL		
Heparinized Whole Blood	150 µL	150 µL	150 µL	30 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				

6.5 Acylation

The Acylation is performed in the Device for Acylation. The reagents and devices for the acylation reaction are mainly integral part of the BA 10-1000 test kit.

	Standards	Controls	Samples
Standards	25 µl		
Controls		25 µl	
Samples			50 µl
Release Buffer	25 µl	25 µl	
Acylation Buffer	25 µl	25 µl	25 µl
Equalizing Reagent	25 µl	25 µl	25 µl
Acylation Reagent	10 µl	10 µl	10 µl
Incubate for **1 hour** at **RT** on a shaker (400-500 rpm)			
Distilled Water	200 µl	200 µl	200 µl
Incubate for **30 minutes** at **RT** on a shaker (400-500 rpm)			
20 µl of the acylated standards, controls and samples are needed for the ELISA			

6.6 Histamine ELISA

The quantification of the Histamine has to be done with the Histamine ELISA test kit (REF BA 10-1000), starting with Chapter 6.3 **Test procedure** on page 4 of the test instructions.

7. Calculation of results

The histamine concentrations of the samples from the release test can be read directly from the standard curve of the Histamine ELISA (BA 10-1000).
The result for the total histamine has to be multiplied by the factor 5.
The histamine concentration from spontaneous release has to be subtracted from the allergen-induced histamine and total histamine concentrations.

8. Quality control

Please refer to the instructions for use of the Histamine ELISA (REF BA 10-1000).

9. Advice on handling the test

Please refer to the instructions for use of the Histamine ELISA (REF BA 10-1000).

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!