DIAGNOSTIKA GMBH

Instruction for use

Histamine ELISA in Plasma

Enzyme Immunoassay
for the Quantitative Determination of
Histamine in Plasma

CE

IVD

REF EA206/96

12 x 8

2 – 8 °C

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October 2011

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1. Introduction and Principle of the Test

Histamine (β-imidazole-ethylamine) a biogenic amine, is a product of the histidine metabolism. It is produced by decarboxylation of histidine.

Histamine is widely distributed in mammalian tissues. It's bound to heparin (as inactive form) and stored in the granules of basophilic leukocytes and mast cells and is actively released as required. These cells, if sensitized by IgE antibodies attached to their membranes, degranulate when exposed to the appropriate antigen.
Histamine plays a major rule in the initial phase of an anaphylactic reaction.
The quantification of histamine in plasma after allergen administration is of clinical interest.

Histamine is part of the immune response to foreign pathogens and it increases the permeability of the capillaries to white blood cells and other proteins, in order to allow them to engage foreign invaders in the affected tissues.

Responsible for the biological effects of histamine in tissue are the activation of different surface receptors, for instance H1, H2 and H3.

Histamine is involved in the regulating physiological function in the gut and acting as a neurotransmitter.

The competitive Histamine ELISA kit uses the microtitre plate format. Histamine is bound to the solid phase of the microtiter plate. Acylated histamine and solid phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase histamine is detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

2. Precautions

- For in vitro use only.
- Disposable gloves and safety glasses should be used.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
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.

4. Contents of the Kit

4.1 **MT-Strips** **STRIPS** 12 strips
8 wells each, break apart
precoated with histamine

4.2 **Standards 1 - 6** **CAL 1-6** 6 vials
Each 4 ml, ready for use
Concentrations:

Standard	1	2	3	4	5	6
ng/ml	0	0.15	0.50	1.5	5	25

4.3 **Control 1 & 2** **CON 1 & 2** 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

4.4 **Acylation Buffer** **ACYL-BUFF** 1 vial
3.5 ml, ready for use

4.5 **Acylation Reagent** **ACYL-REAG** 3 vials
lyophilised, dissolve content
in 1.5 ml Solvent , if necessary
combine the contents of 2or 3 vials (see 6.1.3.)

4.6 **Antiserum** **AS** 1 vial
3 ml, ready for use, colour coded blue
Rabbit-anti-N-acyl-histamine

4.7 **Enzyme Conjugate** **CONJ** 1 vial
12 ml, ready for use
Goat anti-rabbit-IgG-peroxidase

4.8 **Wash Buffer** **WASH** 1 vial
20 ml, concentrated
Dilute content with distilled water to 500 ml total volume.

4.9 **Substrate** **SUB** 1 vial
12 ml TMB solution, ready for use

4.10 **Stop Solution** **STOP** 1 vial
12 ml, ready for use
Contains 0.3 M sulphuric acid

4.11 **Reaction plate** **ACYL-PLATE** 1 piece
for acylation

4.12 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilzed, dissolve content with 12 ml distilled water,
dissolve carefully to minimize foam formation.

4.13 **Solvent** **SOLVENT** 1 vial
5.5 ml solvent to dissolve the Acylation reagent
Contains DMSO and Acetone, ready for use

4.14 **Start buffer** **START-BUFF** 1 vial
6 ml, ready for use

4.15 **Adhesive Foil** **Foil** 1 piece
Ready for use

Additional materials and equipment required but not provided:

- Pipettes (25, 50, 100 and 1000 µl)
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Distilled water

5. Sample Collection

The test can be performed with EDTA plasma.

Hemolytic and lipemic samples should not be used.

The samples can be stored up to 6 hours at 2 - 8 °C. For a longer storage (up to 6 months) the samples must be frozen at -20 °C

Repeated freezing and thawing should be avoided.

6. Preparation of Reagents and Samples

6.1. Preparation of Reagents

6.1.1. Wash Buffer WASH

Dilute the content with dist. water to a total volume of 500 ml.
For further use the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.

6.1.2. Equalizing Reagent EQUA-REAG

Dissolve the content with 12 ml dist. water, mix shortly and leave on a roll mixer for 30 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable for a minimum of 1 year.

6.1.3. Acylation Reagent ACYL-REAG

Dissolve the content of one bottle in 1.5 ml Solvent and shake for 5 minutes on an orbital shaker. The Acylation Reagent has always to be prepared immediately before use. After use the reagent has to be discarded.
The second and third vial allows a second and third run of the test, respectively. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of the three vials of Acylation Reagent.

Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices
Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

All other reagents are ready for use.

6.2. Preparation of Samples (Acylation)

Allow reagents and samples to reach room temperature.
Determinations in duplicates are recommended.

The wells of the reaction plate for the acylation can be used only once. So please mark the respective wells before using.

1. Pipette each 50 µl standard 1 - 6, control 1 & 2 and plasma samples into the respective wells of the reaction plate.

2. Pipette each 25 µl Acylation Buffer into all wells.

3. Pipette each 100 µl Equalizing Reagent into wells. Mix the reaction plate for 10 seconds.

4. Pipette each 25 µl Acylation Reagent into all wells and continue with step 5. immediately.
   Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices
   Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and well by well.

5. Incubate for 60 minutes at room temperature on an orbital shaker. Do not cover the wells or the plate; leave the plate open on the shaker.

   Take each 50 µl for the ELISA.

7. Test Procedure ELISA

Allow reagents and samples to reach room temperature.
Determinations in duplicates are recommended.

1. Pipette each 50 µl Start buffer into all wells.

2. Pipette each 50 µl prepared Standards 1 to 6, controls and Plasma Samples into the respective wells of the coated microtiter strips.

3. Pipette each 25 µl Antiserum into all wells.

4. Cover the plate with adhesive foil, shake for 10 seconds and incubate for 15 – 20 hours (overnight) at 2-8°C.

5. Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl Wash Buffer. Repeat the washing procedure 3 to 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

6. Pipette each 100 µl enzyme conjugate into all wells.

7. Incubate for 60 minutes at room temperature on an orbital shaker.

8. Washing: Repeat step 5.

9. Pipette each 100 µl Substrate into all wells.

10. Incubate for 20 to 30 minutes at room temperature (20 °C - 25 °C) on an orbital shaker.

11. Pipette each 100 µl Stop Solution into all wells.

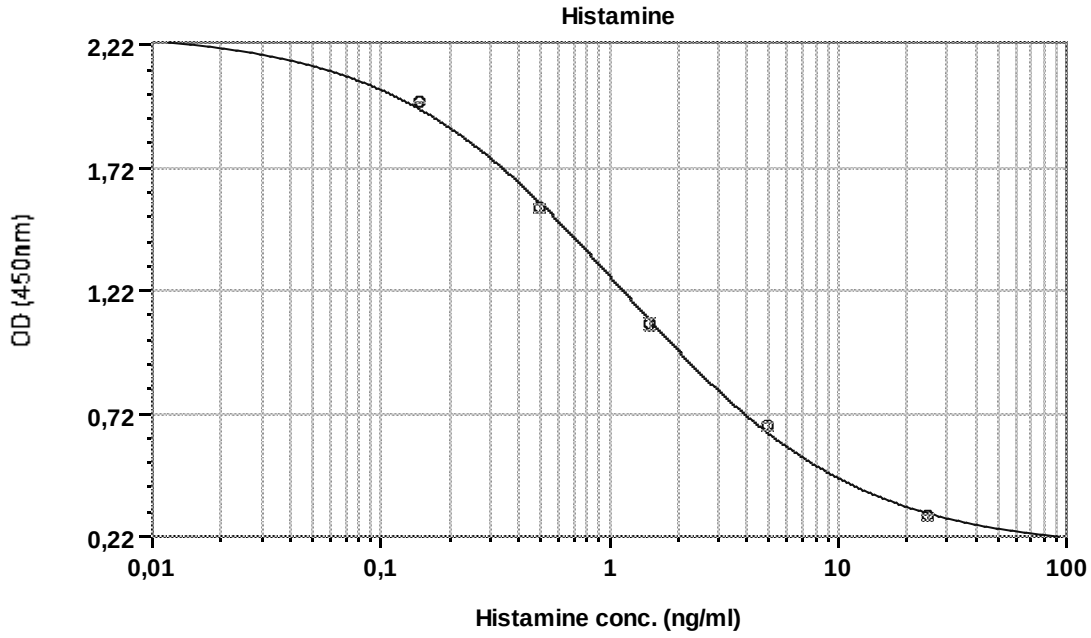
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 10 minutes.

8. Calculation of the Results

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis.

The concentration of the controls and plasma samples can be read directly from this standard curve by using their average optical density.

Typical standard curve:



y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
Std (Standards: Concentration vs MeanValue)	2,272	0,85	1,136	0,171	0,999

9. Assay Characteristics

9.1 Normal Range

The reference range given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Normal range plasma: < 1 ng/ml

9.2 Sensitivity

The lower limit of detection was determined by taking the 3fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

Sensitivity: 0.02 ng/ml

9.3. Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against histamine used in the ELISA method. The tested compounds were 3-Methylhistamine, L-Histidine, Tryptamine and Tyramine.

Substance	ED-50-Value (ng/ml)	**Cross Reactivity (%)**
Histamin	2.0	**100**
3-Methylhistamine	2850	**0.07**
Tyramine	> 100,000	**< 0.002**
L-Histidine	> 1,000,000	**< 0.0002**
Tryptamine	> 1,000,000	**< 0.0002**

9.4. Recovery

Increasing amounts of histamine were added to a plasma sample. Each spiked sample was assayed. The analytical recovery of histamine was estimated at different concentrations by using the theoretically expected and the actually measured values. The mean recoveries from all concentrations were 102%.

Concentrations in ng/ml

added	measured	expected	% recovery
0	0.47		
0.19	0.66	0.65	**100**
0.38	0.92	0.85	**108**
0.57	1.12	1.03	**109**
0.87	1.59	1.34	**118**
1.15	1.85	1.62	**114**
1.43	1.84	1.90	**97**
1.88	2.47	2.34	**105**
2.68	2.83	3.14	**90**
3.85	4.16	4.31	**96**
5.66	4.99	6.13	**81**
8.70	9.71	9.20	**105**
12.5	12.3	12.9	**95**
		mean recovery:	**102**

9.5. Linearity

The linearity of the ELISA method was investigated using different dilutions of a plasma sample. The mean linearity from all dilutions was 105%.

Concentrations in ng/ml

dilution	measured	recalculated value	recovery %
orig.	19.6		
4+1	16.6	15.7	**106**
2+1	12.6	13.1	**96**
1+1	9.8	9.8	**101**
1+2	6.9	6.5	**106**
1+3	5.4	4.9	**111**
1+4	3.9	3.9	**100**
1+6	3.2	2.8	**113**
1+9	1.9	2.0	**96**
1+14	1.4	1.4	**111**
1+19	1.2	0.98	**123**
1+24	0.80	0.78	**102**
1+49	0.48	0.39	**123**
1+99	0.16	0.20	**81**
		mean linearity:	**105**

9.6. Reproducibility

The reproducibility of the ELISA method was investigated by determine the intra-assay-coefficients of variation (cv) by repeated measurements of two plasma samples with different histamine concentrations.

Concentrations in ng/ml

Intra-Assay Variation

sample	n	mean value	sd	cv (%)
1	40	0.47	0.051	**10.9**
2	40	3.1	0.233	**7.6**

10. Literature

- Lichtenstein, L.M., Osler, A.G.
  Studies on the mechanisms of hypersensitivity phenomena IX. Histamine release from human leukocytes by ragweed pollen antigen
  J. Exp. Med., 507-530

- Matsumoto, J., Matsuda, H
  Mast-cell dependent histamine release after praziquantel treatment of Schistosoma Japonicum infection: implications for chemotherapy-related adverse effects
  Parasitol Res 88 (2002), 888-893

- Koller, D.Y., Rosenkranz, A.R., Pirker, C., Götz, M., Janisch, R.
  Assessment of histamine release from basophile in whole blood by benzylpenicilloyl poly-L-lysine in penicillin-sensitized patients
  Allergy, 47, (1982), 459-462

- McBride, P., Bradley, D., Kaliner, M.
  Evaluation of a radioimmunoassay for histamine measurement in biological fluids
  J. Allergy Clin. Immunol., 82, 638-646

- Douglas, W.W.
  Histamine and antihistamines; 5-Hydroxytryptamine and antagonists
  The pharmacological basis of therapeutics (5e ed.) Ed. Goodman, L.S., Gilman, A., MacMillian Publishing Co Inc, N.Y., 590-629

Pipetting Scheme Sample Preparation

		Standards	Control	Plasma Sample
Standard 1 - 6	µl	50		
Control 1 & 2	µl		50	
Plasma Sample	µl			50
Acyl. Buffer	µl	25	25	25
Equalizing Reag.	µl	100	100	100

Shake for 10 seconds

Freshly prepared Acyl. Reagent	µl	25	25	25

Immediately: 60 minutes incubation at room temperature on an orbital shaker
Do not cover wells or plate, leave the plate open on the shaker

take each 50 µl for the ELISA

Pipetting Scheme ELISA

		Standards	Control	Plasma Sample
Start buffer		50	50	50
Standard 1 - 6	µl	50		
Control 1 & 2	µl		50	
Plasma Sample	µl			50
Antiserum	µl	25	25	25

Cover the plate with adhesive foil
Shake for 10 seconds
Incubate for 15 – 20 hours (overnight) at 2-8°C

3 - 4 x washing

Enzyme Conjugate	µl	100	100	100

60 minutes incubation at room temperature on an orbital shaker

3 - 4 x washing

Substrate	µl	100	100	100

20 - 30 minutes incubation at room temperature on an orbital shaker

Stop Solution	µl	100	100	100

Reading of absorbance at 450 nm