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Instructions for use

FD HIS SUB ELISA

HISTAMINE ELISA

stool, urine and whole blood

REF FD E-3000

2°C–8°C Σ 96 IVD CE

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Histamine in stool, urine and whole blood.

First, Histamine is quantitatively acylated. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated analyte concentrations in the standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-goat IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standard concentrations.

1.2 Clinical application

Histamine belongs to the biogenic amines and is synthesized by decarboxylation from the amino acid histidine. It is synthesized by mast cells, basophils, platelets, histaminergic neurons, and enterochromaffine cells, where it is stored intracellularly in vesicles and released on stimulation.
Histamine acts by binding to its 4 receptors (H1R, H2R, H3R and H4R) on target cells in various tissues. It causes smooth muscle cell contraction, vasodilatation, increased vascular permeability and mucus secretion, tachycardia, alterations of blood pressure, and arrhythmias.
In humans, histamine is one of the most important mediators and takes part in the initial phase of an anaphylactic reaction ("immediate type" allergy).
Of clinical interest is also the quantification of the histamine release from basophilic leucocytes in allergies.
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as under point "Procedural cautions, guidelines and warnings". Any laboratory result is only a part of the total clinical picture of the patient.
Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient it can be used for therapeutic consequences.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for certain types of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.

(16) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(17) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(19) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
(20) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

24-hour urine
Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of histamine level in the sample.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

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 D-0024 REAC-PLATE **Reaction Plate** - Ready to use
Contents: 1 x 96 well plate, empty in a resealable pouch

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 ml/vial, light purple cap

BA E-1040 CONJUGATE **Enzyme Conjugate** - Ready to use
Contents: Donkey anti-goat immunoglobulins conjugated with peroxidase
Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 ml/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

BA E-1031 MTP HIS **Histamine Microtiter Strips** - Ready to use

Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable foil pouch with desiccant

BA E-1210 HIS-AS **Histamine Antiserum** - Ready to use

Contents: Goat anti-histamine antibody, blue coloured

Volume: 1 x 12 ml/vial, blue cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/g	Volume/Vial
BA E-1001	STANDARD A	white	0	4 ml
BA E-1002	STANDARD B	light yellow	0.5	4 ml
BA E-1003	STANDARD C	orange	1.5	4 ml
BA E-1004	STANDARD D	dark blue	5	4 ml
BA E-1005	STANDARD E	light grey	15	4 ml
BA E-1006	STANDARD F	black	50	4 ml
BA E-1051	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!	4 ml
BA E-1052	CONTROL 2	dark red		4 ml

Conversion: Histamine (ng/ml) x 9 = Histamine (nmol/l)

Contents: Acidic buffer spiked with defined quantity of Histamine

BA E-1711 ACYL-BUFF **Acylation Buffer** - Ready to use

Contents: TRIS-buffer, red coloured

Volume: 1 x 22 ml/vial, brown cap

BA E-1712 ACYL-REAG **Acylation Reagent** - Ready to use

Contents: Acylation reagent containing DMSO

Volume: 1 x 3 ml/vial, green cap

BA E-0041 DILUENT **Diluent** - Ready to use

Contents: Acidic buffer with non-mercury preservatives

Volume: 1 x 22 ml/vial, white cap

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

- Calibrated precision pipettes to dispense volumes between 20 - 200 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
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

⚠ *The assay can be performed with or without the usage of a microtiter plate shaker. If a shaker is used, it should have the following characteristics: shaking amplitude 3 mm, capable of approx. 600 rpm.*

⚠ *For the determination of total histamine in whole blood, a **Release Buffer** is necessary! (Please ask your local supplier for ordering details)*

BA E-1726 RELEASE-BUFF **Release Buffer** - Ready to use

Contents: Buffer with physiological pH

Volume: 1 x 250 ml/vial, white cap

5. Sample collection and storage

Stool

For the collection of stool samples a special Stool- Collection Device is mandatory **FD Histamine stool tube** REF (FD-ROE-40-GB). We prefer to use a complete mail order approved **FD stool-Collection-shipping box** REF (FD-H-VB). Please ask the manufacture for the device or set. Stabilized samples can be stored one week at RT (20-25°C) or up to 6 months at 2 – 8 °C. The stool collection tube take up 40 mg stool sample and contain 3 ml stabilizer.

Urine
Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, may be used. If 24-hour urine is being used please record the total volume of the collected urine.
Storage: up to 6 hours at 2 – 8 °C; for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.
Avoid exposure to direct sunlight.

Whole Blood
Whole blood is collected into a tube (e.g. Monovette™ or Vacuette™) containing heparin as anti-coagulant (according to manufacturer's instructions). 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.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. t is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.
Storage: 1 month at 2 – 8 °C

Acylation Reagent
The Acylation Reagent has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution.

Whole Blood
For the determination of total histamine in whole blood, dilute heparinized whole blood 1 + 20 with **Release Buffer** (BA E-1726) and incubate for **10 min at 90 °C** (e.g. 50 µl whole blood plus 1 ml Release Buffer)
Cool down the samples for **10 min at 2 – 8°C**.
Centrifuge for 10 min at 700 x g (the brake should be switched-off!)

Histamine Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Sample preparation and acylation

1.	Pipette **100 µl** of **standards**, **controls** and **25 µl** of **stabilized stool samples** *(please refer to chapter 5, sample collection)* and **20 µl** of **urine samples** and **100 µl** of pretreated **whole blood samples** *(refer to 6.1.)* into the respective wells of the **Reaction Plate**.
2.	Add **75 µl** of **Diluent** to the wells with **stabilized stool samples.**
3.	Add **80 µl** of **Diluent** to the wells with **urine samples**.
4.	Add **25 µl** of **Acylation Reagent** *(refer to 6.1)* to all wells.
5.	Pipette **200 µl** of **Acylation Buffer** into all wells.
6.	Incubate **15 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Alternatively without shaker:*** *shake* ***the Reaction Plate*** *shortly by hand and incubate* ***15 min at RT*** *(20 – 25 °C).*
⚠	**Take 25 µl for the subsequent ELISA**

6.3 Histamine ELISA

1.	Pipette **25 µl** of the **acylated standards, controls and samples** into the wells of the **Histamine Microtiter Strips**.
2.	Pipette **100 µl** of the **Histamine Antiserum** into all wells.
3.	Incubate **30 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ***Alternatively without shaking,*** *shake the* ***Histamine Microtiter Strips*** *shortly by hand and incubate for* ***40 min*** *at* ***RT*** *(20 - 25 °C).*
4.	Discard or aspirate the contents of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **10 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ***Alternatively without shaking,*** *incubate for* ***20 min*** *at* ***RT*** *(20 - 25 °C).*
7.	Discard or aspirate the contents of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **Substrate** into all wells.
9.	Incubate for **15 ± 2 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ⚠ ***Alternatively without shaking,*** *incubate for* **15 ± 2 min** *at* ***RT*** *(20 - 25 °C).* ***Avoid exposure to direct sunlight!***
10.	Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
11.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** *(*if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

		Histamine
Measuring range	Controls	0.18 - 50 ng/ml
	Stool	54 - 15000 ng/g
	Urine	0.9 – 250 ng/ml
	Whole blood	3.8 – 1050 ng/ml

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay 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.*

Controls
The concentrations of the **Controls 1 & 2** can be read directly from the standard curve.

Stool samples
The stool collection devices have a capacity of 40 mg of stool. They contain 3 ml of stabilizing buffer. 100 µl of standards and 25 µl of the stabilized stool samples are used.
Therefore, the overall dilution of a stool sample is 1:300 and the read concentrations of the **stool samples** have to be **multiplied by 300.**

Urine samples
The read concentrations of **histamine in urine** have to be **multiplied by 5**.

The total amount of Histamine excreted in urine during 24 h is calculated as following:
µg/24h = µg/l x l/24h

Whole blood samples
The read concentrations of **histamine in whole blood** have to be **multiplied by 21**.

Conversion
Histamine (ng/ml) x 9 = Histamine (nmol/l)

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

Stool	24 hour-urine	Spontaneous urine	Whole blood (total Histamine)
< 600 ng/g	< 45 µg/d	< 45 µg/g creatinine	20 - 200 ng/ml

7.1 Quality control

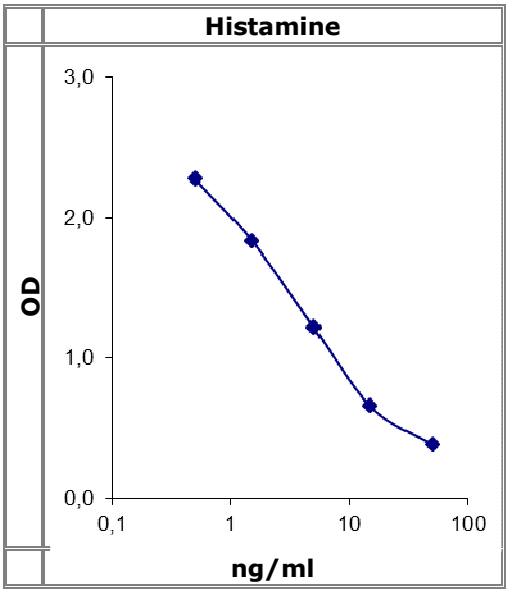
It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are stated in the QC-Report.

7.2 Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

7.3 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

	Substance	Cross Reactivity (%)
Analytical Specificity (Cross Reactivity)	L-Histidine	< 0.001
	L-Tyrosine	< 0.001
	L-Phenylalanine	< 0.001
	Tryptamine	< 0.001
	5-Hydroxy-3Indole Acid	< 0.001
	Serotonin HCl	< 0.001
	Tyramine HCl	0.01
	3-Methyl-Histamine	0.1

Analytical Sensitivity (Limit of Detection)	Stool	75 ng/g
	Urine	0.9 ng/ml
	Whole Blood	3.8 ng/ml

Precision							
Inter-Assay				**Intra-Assay**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Stool	1	15.8 ± 3.4	11.6	Stool	1	15.5 ± 3.2	10.6
	2	54.2 ± 9.0	8.8		2	56.6 ± 18.7	9.4
	3	105 ± 26.3	10.3		3	118 ± 23.7	10.1
Urine	1	27.0 ± 2.5	9.4	Urine	1	27.4 ± 3.1	11.3
	2	30.7 ± 3.2	10.5		2	32.7 ± 3.1	9.5
	3	46.6 ± 4.8	10.3		3	48.2 ± 4.1	8.4
	4	87.2 ± 10.9	12.5		4	88.0 ± 7.0	8.0
Whole Blood	1	52.6 ± 5.3	10.2	Whole Blood	1	48.4 ± 9.1	18.7
	2	74.4 ± 8.9	11.9		2	73.2 ± 8.9	12.1
	3	165 ± 14.1	8.6		3	156 ± 16.6	10.7
	4	426 ± 37.6	8.8		4	383 ± 38.8	10.1

Linearity		**Range (ng/ml)**	**Serial dilution up to**	**Range (%)**
	Stool	19.3 - 31.6	1:64	92 - 115
	Urine	17.2 – 53.3	1:64	89 - 112
	Whole Blood	39.9 – 71.4	1:64	81 - 105

Recovery		**Range (ng/ml)**	**Mean (%)**	**Range (%)**
	Stool	5.2 - 137	96	82 - 120
	Urine	22.6 – 235	95	86 – 100
	Whole Blood	56.7 - 1058	106	87 - 115

Method Comparison with LC-MS/MS	Urine	LC-MS/MS = 1.2 ELISA – 27.0	r = 0.92	n = 40
	Whole Blood	LC-MS/MS = 0.8 ELISA – 6.8	r = 0,98	n = 40

9. References/Literature

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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		

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