BioVendor Group

Product Data Sheet:

HISTAMINE ELISA

ENG

Catalogue number:

RA19008R

For research use only!

BioVendor R&D

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CONTENTS

1. HISTAMINE ELISA

96 wells

Storage: -20°C

Expiry date: stated on the package

This kit contains:

REAGENTS	Quantity	Form
Antibody Coated Microtiter Strips (with mouse anti-Histamine monoclonal antibody, ready to use after thawing)	1	
Conjugate Solution (Histamine tracer)	1	lyophilized
Histamine Standard	2	liquid
Derivatization reagent	2	powder
Derivatization buffer	1	liquid ready to use
Quality Control sample	2	liquid
Dilution Buffer (EIA buffer)	1	lyophilized
Wash Solution Concentrate	1	liquid
Substrate Solution (Ellman´s reagent)	2	lyophilized
Tween 20	1	liquid
Cover Sheet	1	
Template Sheet	1	

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 33 samples in duplicate.

2. PRECAUTION FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample or reagent and dispense it back into the same vessel.

Repeat this operation two or three times before distribution.

- For research laboratory use only.
- Not for diagnostic use.
- Do not pipet liquids by mouth.
- Do not use kit components beyond the expiration date.
- Do not eat, drink or smoke in area in which kit reagents are handled.
- Avoid splashing.

The total amount of reagents contains less than 100 µg of sodium azide. Flush the drains thoroughly to

prevent the production of explosive metal azides.

3. PRINCIPLE OF THE ASSAY

This Enzyme Immunoassay (EIA) is based on the competition between unlabelled derivatized histamine and acetylcholinesterase (AChE) linked to histamine (tracer) for limited specific mouse anti-histamine antibody sites.

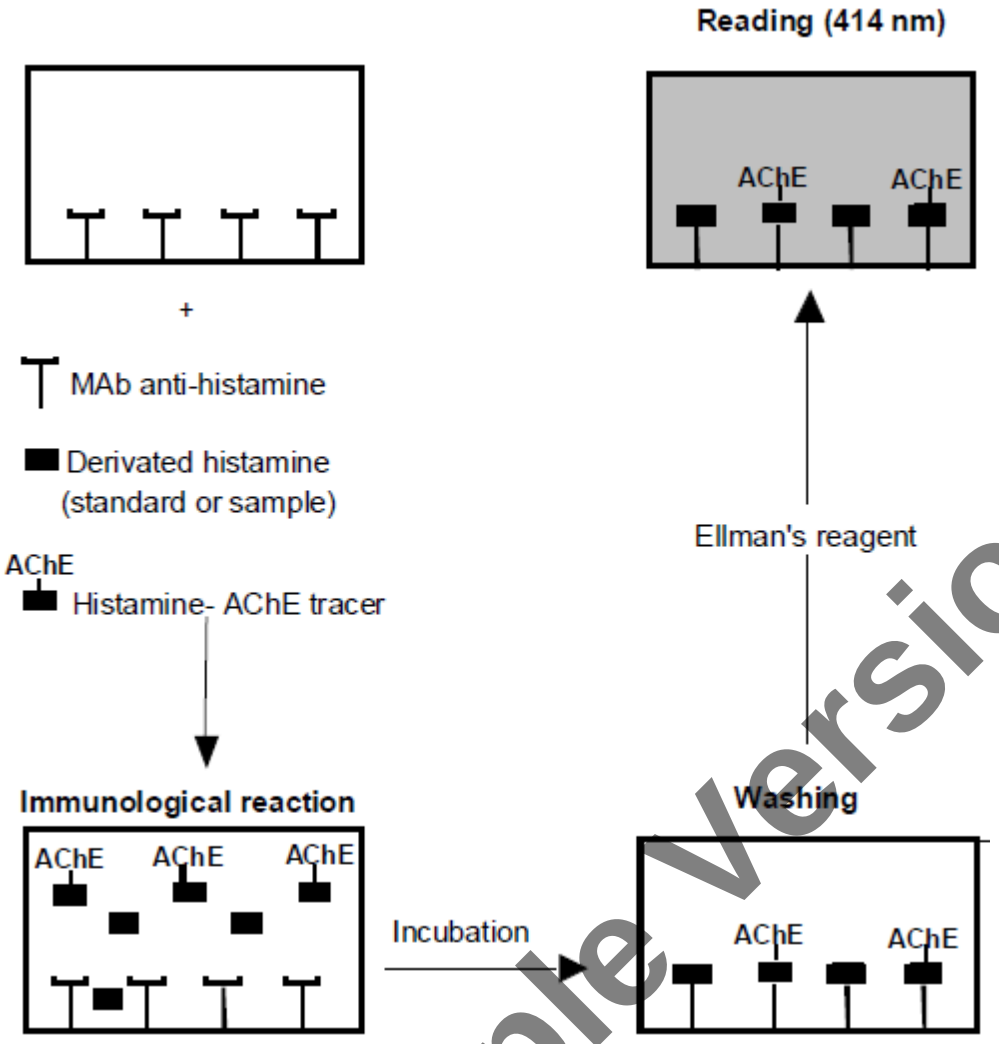
As a former step of this assay, histamine is derivatized to increase the affinity of histamine to the antibody and consequently increase the sensitivity of the assay.

Tracer and standard (or sample) are incubated in wells which have been precoated with a mouse antihistamine antibody attached to the well. The plate is washed to remove any unbound reagent, and Ellman's Reagent (enzymatic substrate for AChE and chromogen) is added to the wells.

The AChE tracer acts on the Ellman's Reagent to form a yellow compound that strongly absorbs at 412 nm.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free histamine present in the well during the immunological incubation.

The principle of the assay is summarised as follows:



4. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following material is required:

- N-N-Dimethylformamide (DFM)
- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (405 or 414 nm filter)
- Microplate washer (or wash-bottles)
- Distilled or deionized water
- Polypropylene tubes (no glass tubes)

5. SAMPLE COLLECTION AND PREPARATION

This assay may be used to measure histamine in samples such as plasma, urine, culture supernatants as well as liquid (e.g. broncho-alveolar lavage fluids) or solid (brain, nervous tissus) biological samples after extraction. Please refer to the appropriate paragraph for your samples preparation protocol.

Blood Sampling

Collect blood samples in tubes containing EDTA. Centrifuge the samples at 1,600 g for 20 minutes. Collect plasma and keep at -20°C until assay. Thaw the samp le on the assay day, vortex and centrifuge it at 1,600 g for 20 minutes, to eliminate fibrin.

Plasma

No prior extraction procedure is necessary to measure histamine in plasma samples. If necessary, plasma samples may be diluted in Histamine EIA buffer before derivatization (see below).

Urine or Culture supernatants

Collect samples in polypropylene tubes. Store the samples at -20°C until assay. No prior extraction is necessary to measure histamine in such samples.

Liquid or solid biological samples

To measure histamine in liquid or solid samples, extract histamine with 0.1 M perchloric acid ($HClO_4$).

Neutralize supernatant fluid with NaOH and apply the derivatization protocol as described below.

6. REAGENT PREPARATION

The coated plates and reagents are provided ready to use.

Dilution Buffer (EIA Buffer)

Reconstitute one vial with 25 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 week.

Histamine Standard

The standard preparation depends of the sample to be assayed:

- for plasma or urine samples, prepare the standard using the Histamine EIA buffer,

- for culture supernatant samples, prepare the standard using the same culture medium as for the sample,

- for extracted liquid or solid samples, prepare the standard in 0.1M $HClO_4$.

In one of standard vial, add 900 µL of assay medium (Histamine EIA buffer, culture medium, $HClO_4$): standard S0 (500 nM). Then dilute 100 µL of S0 in 900 µL of assay medium. The concentration of this standard (S1) is 50 nM. Prepare seven polypropylene tubes (for the seven other standards) and add 500 µL of assay medium into each tube. Add 500 µL of the S1 standard to the first tube and vortex.

Continue this procedure for the other tubes. Thus, standard concentrations are: 50 (S1), 25 (S2), 12.5 (S3), 6.25 (S4), 3.13 (S5), 1.56 (S6), 0.78 (S7) and 0.39 nM (S8), respectively. Stability at 4°C: 1 day.

Quality Control

In one of Quality Control vial, add 900 µL of assay medium, as for the standard. Then dilute 100 µL of QC in 900 µL of assay medium. The final concentration of this QC is labelled on the vial. Stability at 4°C: 1 day.

Derivatization reagent

Before use, reconstitute one vial with 1 mL of N-N-dimethylformamide (DMF). Vortex the contents until completely dissolved. This reagent can not be stored. Eliminate the remaining volume.

Wash Buffer

Dilute 1 mL of the concentrated Wash buffer to 400 mL with distilled or deionized water. Add 200 µL of tween 20 (use a magnetic stirrer to mix the contents). Tween 20 is a viscous liquid and cannot be measured with a pipet. A positive displacement device such as syringe should be used to deliver small quantities accurately. Stability at 4°C: 1 week.

Conjugate Solution

Reconstitute one vial with 10 mL of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 month.

Substrate Solution (Ellman's Reagent)

Five minutes before use, reconstitute with 50 mL of distilled or deionized water. The tube contents should be thoroughly mixed. Stability at 4°C and in the da rk: 4 days.

7. ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

Derivatization of standards, quality controls and samples

The assay procedure depends of the sample to be assayed:

Histamine EIA buffer, plasma, urine, culture supernatant:

In a polypropylene tube, distribute using a pipet:

200 µL of standard, quality control or sample

50 µL of derivatization buffer

In two polypropylene tubes that will allow evaluation of maximum binding (Bo), distribute using a pipet:

200 µL of assay medium

50 µL of derivatization buffer

Vortex all the tubes. Add 20 µL of the derivatization reagent to each polypropylene tube and **vortex each immediately.**

Liquid or solid biological sample:

In a polypropylene tube, distribute using a pipet:

200 µL of standard, quality control or sample

20 µL of 1.5M NaOH

50 µL of derivatization buffer

In two polypropylene tubes that will allow evaluation of maximum binding (Bo), distribute using a pipet:

200 µL of assay medium

20 µL of 1.5M NaOH

50 µL of derivatization buffer

Vortex all the tubes. Add 20 µL of the derivatization reagent to each polypropylene tube and **vortex each immediately.**

Plate preparation

After derivatization, open the plate packet and select the sufficient number of strips for your assay, place the unused strips back in the packet (stored at 4°C). Rinse each well five times with wash buffer (300 µL/well).

Just before distributing reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops.

Distribution of reagents and samples

A plate set-up is suggested on the following page. The contents of each well should be recorded on the sheet provided with the kit.

Pipetting the reagents

Note that the first column should be left empty for blanking Ellman's reagent.

All samples and reagents must reach room temperature prior to performing the assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B_0	S3	S7	*	*	*	*	*	*	*	*
B	B	B_0	S3	S7	*	*	*	*	*	*	*	*
C	B	B_0	S4	S8	*	*	*	*	*	*	*	*
D	B	B_0	S4	S8	*	*	*	*	*	*	*	*
E	B	S1	S5	CQ	*	*	*	*	*	*	*	*
F	B	S1	S5	CQ	*	*	*	*	*	*	*	*
G	B	S2	S6	*	*	*	*	*	*	*	*	*
H	B	S2	S6	*	*	*	*	*	*	*	*	*

B : Blank

S1-S8: Standards 1-8

B_0 : Maximum Binding

* : Samples or Quality contols

Use different tips to pipet the buffer, standard, sample, tracer and other reagents.

- Dispense 100 µL of the derivatized assay medium to Maximum Binding (Bo) wells.

Histamine Standard

Dispense 100 µL of each of the eight derivatized standards (S1 to S8) in duplicate to appropriate wells. Start with the lowest concentration standard (S8) and equilibrate the tip in the next higher standard before pipetting.

Quality Control and samples

Dispense 100 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in Histamine EIA buffer (plasma, urine) or in assay medium (cell culture medium, $HClO_4$…).

Conjugate Solution (Histamine tracer)

Dispense 100 µL to each well except Blank (B) wells.

Incubating the plate (second step)

Cover the plate with a plastic film and incubate for 24 hours at 4°C.

Developing and reading the plate

Reconstitute the wash buffer and Ellman's Reagent as indicated in the reagent preparation section. Empty the plate by turning over and shaking. Wash each well five times with the wash buffer (300 µL/well).

Dispense 200 µL of Ellman's Reagent to each well including blank wells. Incubate in the dark at room temperature. Optimal development is obtained using an orbital shaker. The plate should be read between 405 and 414 nm (yellow colour) when the Maximum Binding (Bo) wells reach an absorbance of 0.2-0.8 unit.

Enzyme Immunoassay Protocol			
Steps	Blank	Maximum binding	Standard & Sample
Step 1: Derivatization	–	200 µL of assay medium	200 µL of standard or sample
	–	20 µL of 1.5M NaOH if assay medium is $HClO_4$	
	–	50 µL of derivatization buffer	
	Vortex all tubes		
	–	20 µL of derivatization reagent	
	Vortex each tube immediately after adding the derivatization reagent		
Wash the plate 5 times			
Step 2: Distribution of reagents	–	100 µL of derivated solution	
	–	100 µL of tracer	
Cover the plate, incubate at 4°C for 24h			
Wash the plate 5 times			
Step 3: Developing	200 µL of Ellman's reagent		
Incubate the plate with an orbital shaker in the dark at room temperature			
Read the plate between 405 and 414 nm			

8. DATA ANALYSIS

Make sure that your Plate Reader has subtracted the absorbance readings of the blank wells (absorbance of Ellman's reagent) from the absorbance readings of the rest of the plate. If not, do so now.

- Calculate the average absorbance for each Bo, standards and samples.
- Calculate the B/Bo (%)or each standard and sample: (average absorbance of standards or sample divided by average absorbance of Bo) & multiplied by 100.
- Using a semi-log graph paper, plot the B/Bo (%) for each standard point (y axis) versus the concentration (x axis). Draw a best-fit line through the points.
- To determine the concentration of your samples, find the B/Bo (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample. Samples with a concentration greater than 50 nM should be re-assayed after dilution in assay medium (cell culture medium, $HClO_4$…).
- Most plate readers are supplied with curve-fitting software capable of graphing this type of data (log/logit or 4-parameter). If you have this type of software, we recommend using it. Refer to the software manual for further information.

9. ACCEPTABLE RANGE

- Bo absorbance: > 200 mAU in the conditions indicated above.
- Sensitivity or 50% B/Bo : less than 10nM (for standard in Histamine EIA buffer).
- QC sample: see the Quality Control Sheet; acceptable range ± 20% of QC value on the Quality Control Sheet

10. TYPICAL RESULTS

Example data

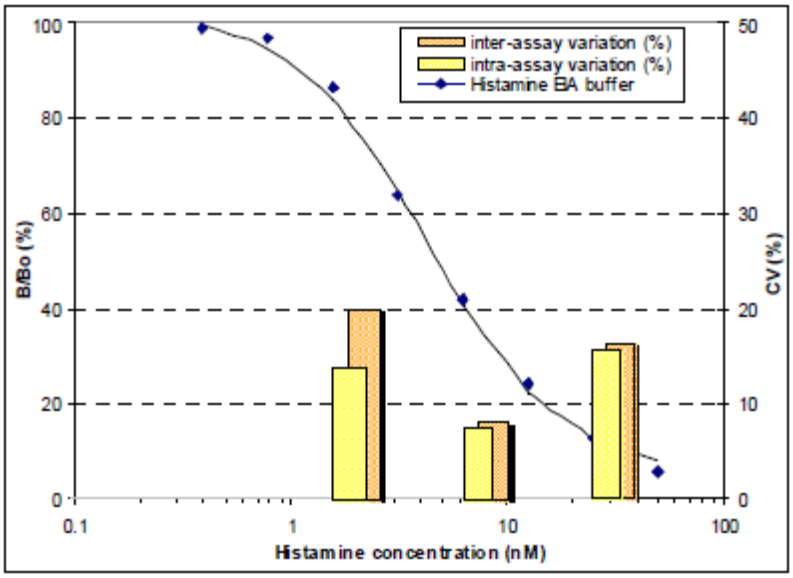
The following data are for demonstration purposes only. Your data may be different but still be correct.

These data were obtained using all reagents as supplied in this kit under the following conditions: 1 hour

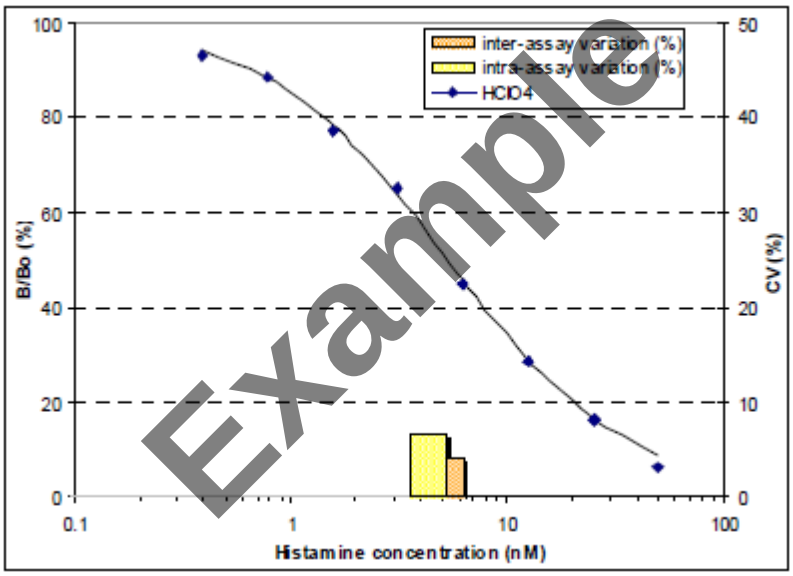
development at 20°C, reading at 414 nm. A 4-paramet er curve fitting was used to determine the concentrations.

Standard or QC	Histamine EIA buffer		Cell culture medium (RPMI)		Perchloric acid ($HClO_4$)	
	mAU	B/Bo (%)	mAU	B/Bo (%)	mAU	B/Bo (%)
Bo	515	100	514	100	321	100
50 nM	30	5.80	45	5.80	21	5.80
25 nM	65	12.6	83	12.6	52	12.6
12.5 nM	124	24.1	148	24.1	92	24.1
6.25 nM	215	41.7	231	41.7	145	41.7
3.13 nM	329	63.8	323	68.8	210	63.8
1.56nM	446	86.6	399	86.6	248	86.6
0.78 nM	499	96.7	414	96.7	285	96.7
0.39 nM	515	99.8	453	99.8	300	99.8
QC 4 nM	249	48.3				

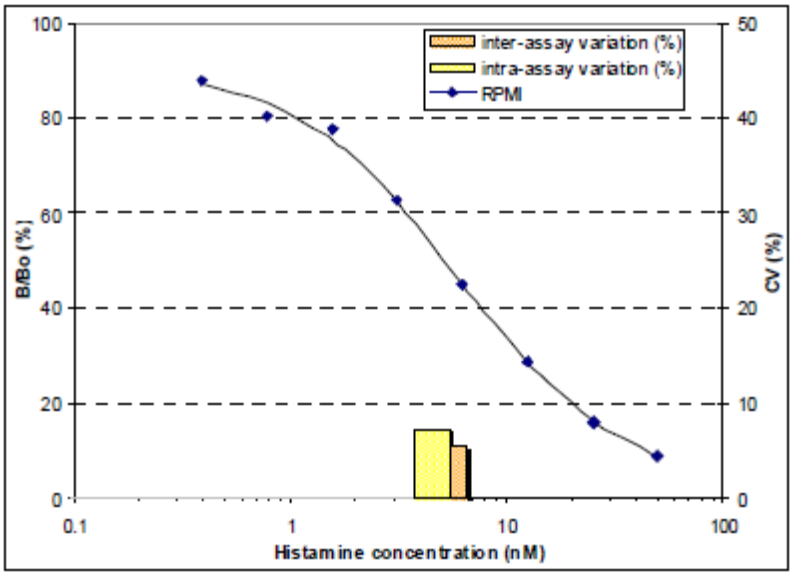
Standard Curve in Histamine Dilution Buffer



Standard Curve in perchloric acid ($HClO_4$)



Standard Curve in cell culture medium (RPMI):



11. ASSAY VALIDATION AND CHARACTERISTICS

Cross-reactivity:

- histamine: 100 %
- histidine: < 0.01 %
- 1 methyl histamine: 0.01 %
- 3 methyl histamine: 0.038%
- serotonin: < 0.01%

Limit of detection:

The limit of detection calculated as the concentration of histamine corresponding to the Bo average minus three standard deviations: 0.5 nM

Intra and Inter-Assay :

	Plasma QC (2.2 nM)	Plasma QC (8.6 nM)	Plasma QC (33.6 nM)
Mean value	_	7,8	32,6
Number of values	30	30	30
Intra-assay coefficient of variation (%)	13,6	7,4	15,1
Inter-assay coefficient of variation (%)	19,4	8,0	15,8
Recovery (%)	_	90,7	97,1
Confidence intervalle	_	90.71 ± 2.74	97.08 ± 5.78

	Buffer QC (1 nM)	Buffer QC (5 nM)	Buffer QC (30 nM)
Mean value	1,2	5,2	32,8
Number of values	30,0	30,0	30,0
Intra-assay coefficient of variation (%)	16,3	9,8	21,4
Inter-assay coefficient of variation (%)	28,9	11,6	21,4
Recovery (%)	123,2	103,4	109,5
Confidence intervalle	123.15 ± 13.39	103.41 ± 4.51	109.47 ± 8.80

	Cell culture medium QC (5nM)	$HClO_4$ QC (5nM)
Mean value	4.58 nM	5.47 nM
Number of Values	20	20
Intra-assay coefficient of variation (%)	7.07%	6.43%
Inter-assay coefficient of variation (%)	5.20%	3.97%
Recovery	91.60%	109.40%

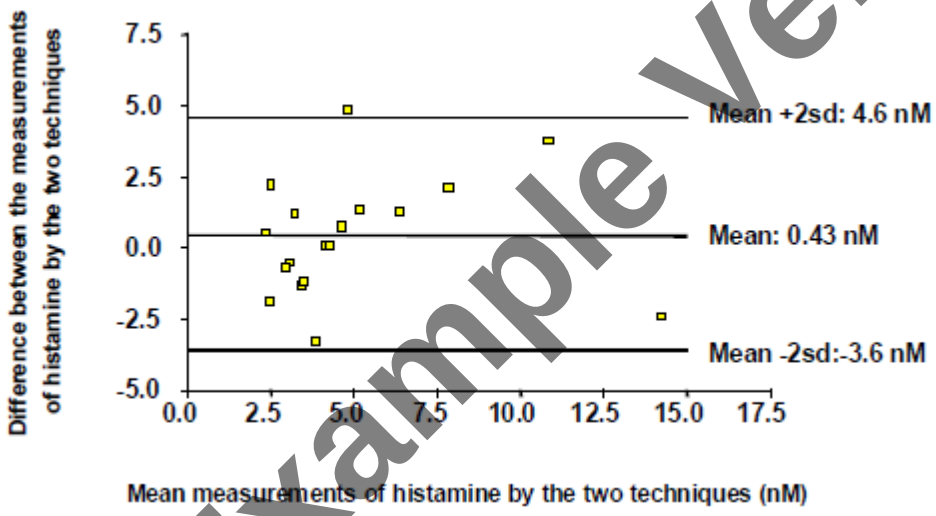
Dilution test:

Day	Dilution factor	Histamine measured	Corrected concentrations	Recovery (%)	Mean
1	1/1	30.79	30.79	94.39	136.41
	1/5	9.40	47.00	144.08	
	1/10	5.08	50.80	155.73	
	1/20	2.47	49.40	151.44	
2	1/1	32.15	32.15	98.56	113.65
	1/5	8.59	43.00	131.82	
	1/10	3.45	34.56	105.95	
	1/20	1.92	38.58	118.27	
3	1/1	31.94	31.94	97.92	130.08
	1/5	8.77	43.83	134.37	
	1/10	4.43	44.31	135.84	
	1/20	2.48	49.65	152.21	
4	1/1	26.56	26.56	81.42	115.24
	1/5	8.17	40.85	125.23	
	1/10	3.86	38.58	118.27	
	1/20	2.22	44.38	136.05	
5	1/1	22.15	22.15	67.90	107.72
	1/5	7.10	35.50	108.83	
	1/10	3.75	37.50	114.96	
	1/20	2.27	45.40	139.18	

Recovery test:

Samples	Histamine added (nM)	Histamine measured (nM)	Recovery (%)
Histamine EIA buffer	5	5.2	103
Cell culture medium	5	5.04	101
Cell culture medium and additives	5	4.58	91.6
$HClO_4$	5	5.47	109

Comparison with reference method on 18 samples:



Stability test (freezing/thawing)

	Plasma CQ1		Plasma CQ2	
	32.6 nM	Recovery (%)	7.8nM	Recovery (%)
1 Cycle	26.69	81.9	8.49	108.9
2 Cycles	29.42	90.3	9.63	123.5
3 Cycles	30.76	94.4	8.22	105.4
4 Cycles	24.26	74.4	6.73	86.3
5 Cycles	-	-	7.78	99.7
Mean	27.78		8.17	
Standard deviation	2.90		1.06	
CV (%)	10.42		12.93	

	Buffer CQ1		Buffer CQ2		Buffer CQ3	
	30 nM	Recovery (%)	5 nM	Recovery (%)	1 nM	Recovery (%)
1 Cycle	36.67	122.2	6.88	137.6	1.36	136.0
2 Cycles	29.63	98.8	4.17	83.4	1.29	129.0
3 Cycles	34.30	114.3	5.62	112.4	1.83	183.0
4 Cycles	29.83	99.4	5.69	113.8	1.31	131.0
5 Cycles	30.17	100.6	6.94	138.8	1.84	184.0
Mean	32.12		5.86		1.53	
Standard deviation	3.19		1.13		0.28	
CV (%)	9.93		19.36		18.56	

12. ASSAY TROUBLE SHOOTING

- Bo value is too low: incubation in wrong conditions (time or temperature) or reading time too short or Histamine-AChE tracer or Ellman's reagent have not been dispensed.
- High dispersion of duplicates: poor pipetting technique or irregular plate washing.
- IC50 or QC concentrations not within the expected range: wrong preparation of standards.
- Analyses of two dilutions of a biological sample do not agree: Interfering substances are present. Sample must be purified prior to EIA analysis (excepting plasma samples).

13. BIBLIOGRAPHY

- **Grassi J. & Pradelles Ph.**

Compounds labelled by the acetylcholinesterase of *Electrophorus Electricus*. Its preparation process and its use as a tracer or marquer in enzymo-immunological determinations.

United States patent, N° 1,047,330. September 10, 1991

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