DRG

DRG® D-HIT (EIA-4599)

CE

RUO in the USA

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1 INTRODUCTION

Histamine is a simple chemical substance with a molecular weight of 111 Da. It is the most important mediator of allergic diseases like rhinitis allergica (hay fever) and asthma bronchiale. Furthermore histamine is the major cause for urticaria and is an important mediator of drug allergy [1,2].

In case of histamine-intolerance various physiological reactions as dilatation of blood vessels or contraction of the uterus are seen. Furthermore, irritating effects like headache, engorged respiration, dripping nose, airway obstruction, tachycardia, extra systoles and gastro intestinal-ailments, which can cause soft stool up to diarrhea and hypotension. Often tumescence of eyelids and sometimes urticarial exanthema were described as well [3, 4, 5, 6, 7].

Histamine is produced within the body and stored in mast cells for immediate release. Additionally histamine is either inhaled (histamine-provocation) or ingested with food containing high levels of histamine or other biogenic amines[8, 9].

The main enzyme for metabolism of ingested histamine is diamine oxidase (DAO). The copper-containing enzyme with a molecular mass of 180 kDa is produced predominantly in the enterocytes. Mainly the enzyme is found in the small intestine, the liver, the kidney and in white blood cells.

The occurrence of different isoenzymes is reported. Nevertheless, few is known about expression and regulation of these isoforms, also contradictive date regarding substrate specificity towards histamine, putrescine and other diamines are found. Possibly a variation in isoenzyme-population also influences the capability of histamine-degradation in the body and therefore causes histamine-intolerance [10, 11, 12, 13, 14, 15, 16, 17].

During pregnancy huge amounts of the enzyme are produced in the placenta, resulting in up to 500 to 1000 fold higher concentrations of the enzyme in the circulation. Because of these high levels of DAO no allergic problems normally are seen in women in the time of 3rd to 9th month of pregnancy[18].

DAO is produced continuously and secreted into the intestine. Therefore most of the histamine ingested with food normally is metabolized immediately within the bowel[19].

Remaining histamine is removed by DAO present in the mucous membrane. It is degraded to imidazole acetaldehyde and further oxidized to imidazole acetic acid. Postulated cofactors of DAO are 6-hydroxy-dopa and vitamin B6 [20, 21, 22, 23, 24]. Diamine oxidase is a rather sensitive enzyme, it is inhibited by other biogenic amines, ethanol, it's degradation product acetic aldehyde and by many different drugs[25, 26].

1.1 Clinical implication

Histamine intolerance is defined by an imbalance of histamine and the histamine degrading enzyme diamine oxidase, which is mainly produced in the small intestine. The present clinical experiences show that histamine intolerance is not congenital, but rather an acquired disease. A cause of histamine intolerance (HIT) may be drugs, which are inhibitors of the diamine oxidase. Alcohol and its degradation product acetaldehyde are inhibitors too. Additionally yet unknown effects on the activation of isoforms may have influence on the histamine-degrading capacity.

The typical symptoms of histamine intolerance are headache, diarrhea, migraine, engorged or dripping nose and especially in connection with food incorporation asthma bronchiale and arrhythmia, hypotension, urticaria and dysmenorrhea[27, 28, 29]. The diagnosis of histamine intolerance is achieved by thorough anamnesis and by the determination of the diamine oxidase activity in plasma or serum[30, 31].

Following a histamine-free diet normally results in a significant reduction or even disappearance of the symptoms within a few weeks.

Because of the increased operation risk of histamine intolerant patients and the typical intolerance to roentgen contrast agent (histamine-liberator) and antirheumatica, histamine intolerance, which has a prevalence of 2-3% of the population, must be of special interest for all physicians. Especially anaphylactic shocks after bites from wasps or bees are often observed in combination with histamine intolerance.

The diagnosis of histamine intolerance based on the activity of diamine oxidase hence is only admissible when the activity is low. Nevertheless, when normal DAO activity and simultaneously increased histamine-levels are found, symptoms of histamine intolerance may be present. Especially after extreme histamine exposure like an anaphylactic shock increased activity of diamine oxidase and elevated histamine levels were measured.

Therefore the interpretation of the diamine oxidase activity always has to be done in context with thorough anamnesis of the patients. A systematic provocation with histamine in a clinically controlled environment can give more details on the ability of the organism in degrading histamine. However, a time-course of DAO-activity gives best data for the diagnosis of histamine intolerance.

Patients suffering from diseases like Urticaria, Morbus Crohn or Celiac Disease are reported to show low DAO activity in serum or plasma[32, 33]. Furthermore DAO is described as marker for the status of the gut mucosa – patients with chronic gut failure often show have reduced DAO values[34].

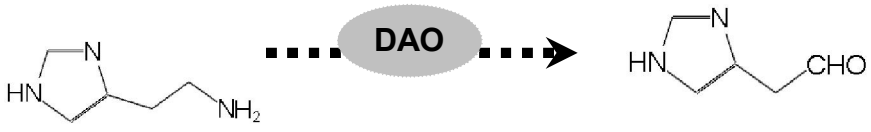
2 PRINCIPLE OF THE ASSAY

50 µL of human serum and EDTA-plasma may be used as analyte.

For activity calculation in plasma a correction factor must be used !

With DAO active ELISA activity of diamine oxidase is determined for the first time by degradation of histamine. The result is given in HDU (histamine degrading units) per mL. 1 HDU corresponds to the activity of DAO that degrades 1 pmol/ml (0.11 ng/ml) of histamine.
All assays used until now used radiolabelled putrescine as a substrate for the enzyme. Activity towards Histamine and Putrescine are different[35, 36.]

In the first step 50 µL of sample are pipetted in a microwell plate containing histamine. During the incubation over night at room temperature (18-26°C) histamine is degraded by DAO present in the sample.



In the next step the remaining histamine is acetylated.

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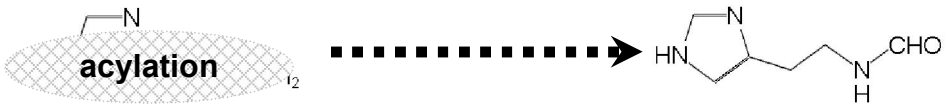


Part of the reaction mixture is transferred into the ELISA plate and Antibody is added.

Acylated histamines and coated acyl histamine compete for the binding sites of the antibody.

All unbound substances are removed in a washing step.

The amount of antibody bound is detected using a HRPO-labelled secondary antibody (Conjugate). The signal is directly proportional to the activity of DAO in the sample.

3 CONTENTS OF THE KIT

1. **Standards**
   The 6 vials contain diamine oxidase (DAO, EC 1.4.3.6.) from porcine kidney, lyophilized.
   The actual concentration is stated on the label and on the QC-protocol
2. **Control**
   The vial contains diamine oxidase (DAO, EC 1.4.3.6.) from porcine kidney, lyophilized.
   The actual concentration is stated on the label and on the QC-protocol
3. **Incubation Plate** (marked blue). The wells contain lyophilized histamine
4. **Antibody,** lyophilized
5. **ELISA-Plate**. The single break apart wells are coated with acylated histamine
6. **Assay Reagent**, 35 mL, ready to use
7. 4 vials **Acylation reagent**, lyophilized
8. **Acylation Diluent,** 5 mL, for dissolving the Acylation reagent
9. **Assay Buffer,** 30 mL, ready to use
10. **10x Wash Buffer concentrate,** 60 mL
11. **Conjugate,** 13 mL, ready to use
12. **Substrate,** 13 mL, ready to use
13. **Stop Solution,** 7 mL, ready to use
14. Instruction manual
15. 4 **sealing tapes**
16. Quality Control Protocol of the respective batch
17. Protocol sheet

4 ADDITIONAL MATERIAL AND EQUIPMENT REQUIRED

- Precision pipettes in the range of 20 to 1000 µL,
- Multichannel pipette
- Multipette
- Incubator 37°C (preferably shaker-incubator 37°C)
- ELISA-reader with filter 450 nm (optional: 620 nm)
- Graph paper or software for calculation of results

5 PERFORMANCE OF THE ASSAY

Freshly collected samples (serum or EDTA-plasma) may be stored at 4°C up to 48 hours, for prolonged storage samples must be frozen at –20°C.

Lipemic or hemolytic samples may give erroneous results.

Samples should be mixed well before assay performance.

We recommend duplicates for all determinations.

Day 1:

1. Take needed microtiter strips of **Incubation Plate (marked blue)** out of the foil pouch – appearance of the wells can vary – this has not influence on assay quality ,
   store unused strips sealed with plastic in the foil pouch with desiccant at 4°C (Silicagel in desiccant bag should stay orange, otherwise plate has to be thrown away)
2. Dissolve **Standards** in 0.5 mL **Assay Reagent**; leave for 15 min at RT (18-26°C), mix well (Vortex). *Store unused standards up to 3 days at 4°C, for prolonged storage freeze at -20°C.*
3. Dissolve **Control** in 0.5 mL **Assay Reagent**; leave for 15 min at RT (18-26°C), mix well (Vortex). *Store unused control up to 3 days at 4°C, for prolonged storage freeze at -20°C.*
4. Mark wells for standards and samples on the protocol sheet.
5. Add 50 µL of **Assay Reagent** to each well, mix gently
6. Incubate strips for 30 min at room temperature (18-26°C), **DO NOT SEAL**.
7. Add each of 50 µL **Standard, Control** and **Sample** into respective wells of **Incubation Plate** (marked blue).
8. Cover strips and incubate over night at 37°C, shake if possible at 400 rpm.
   Do use only the foil packed in the kit!
   To avoid desiccation of the wells seal perfectly!

Day 2:

9. Dilute **10 x Wash buffer concentrate** with aqua deion. 1 + 9
   (60 mL washing buffer concentrate plus 540 mL aqua deion.)
   Diluted washing buffer is stable at 4°C until expiry date stated on the label.
10. Reconstitute **Antibody** in 20 mL of **Assay Buffer.**
    Store unused antibody up to 3 days at 4°C, for prolonged storage freeze at -20°C.
11. Reconstitute each vial of **Acylation Reagent** with 1 mL of **Acylation Diluent**, mix well.
    NOTE: If more than one vial is used, pool the contents and mix well.
    (Acylation Diluent is an organic solvent and should be pooled in glass bottles only. ATTENTION: Removes labelling from Multipette tips – do not soak tip in solution!)
    Use reconstituted reagent within 30 minutes, it cannot be stored.
12. Carefully add 20 µL of reconstituted **Acylation Reagent** in every well of **Incubation Plate** (marked blue) containing the sample (Multipette). To avoid drift effects pipette whole plate within 5 minutes!

13. Incubate strips for 30 min at 37°C (shake if possible at 400 rpm), ***DO NOT*** cover with sealing tape!
14. Add 150 µL **Assay Reagent** to all wells.
15. Incubate strips for 30 min at 37°C (shake if possible at 400 rpm), ***DO NOT*** cover with sealing tape!
16. Take microtiter strips of **ELISA Plat** needed **e** out of the foil pouch,
    store unused strips sealed with plastic in the foil pouch with desiccant at 4°C (Silicagel in desiccant bag should stay orange, otherwise plate has to be thrown away).
17. Transfer 30 µL of reaction mixture from **Incubation Plate** (marked blue) into respective wells of **ELISA Plate** (use Multi channel pipette). Gently mix the contents of the Incubation plate well with the pipette before transfer).
18. Add 150µl of dissolved **Antibody** into each well (as described in 10.).
19. Cover strips and incubate for 45 min at 37°C (shake if possible at 400 rpm).
20. Discard contents of wells and wash 4 times with 300 µL of **diluted wash buffer**.
21. Pipette 100 µL of **Conjugate** into all wells.
22. Cover strips and incubate for 30 min at 37°C on a shaker-incubator (at 400 rpm).
23. Discard contents of wells and wash 4 times with 300 µL of **diluted wash buffer.**
24. Add 100 µL of **Substrate** into all wells.
25. Incubate for 15 min at room temperature (18-26°C).
26. Add 50 µL of **Stop Solution** into all wells.
27. Read absorbance at 450 nm in a ELISA-Reader (user 620 nm as reference).

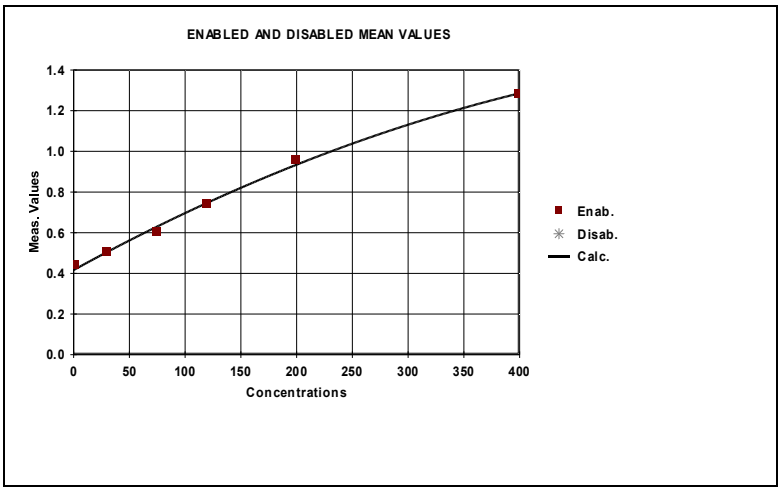
6 CALCULATION

Construct the Standard curve from the Standard values. Use commercially available software or graph paper. Obtain sample concentration from this Standard curve.

ATTENTION:
Results of plasma samples have to be multiplied with the factor 1,25 to adjust for matrix effects.

Target concentration of Control is stated on the label!

The graph shows an example of calibration curve.



THIS GRAPH MUST NOT BE IMPLIED FOR THE CALCULATION OF MEASURED VALUES!

7 TEST CHARACTERISTICS

7.1 Reference values

DAO > 80 HDU/ml: normal activity of DAO
DAO 40-80 HDU/ml: reduced activity of DAO
DAO < 40 HDU/ml: highly reduced activity of DAO

It is IMPOSSIBLE to state histamine intolerance in case of anaphylactic shock and during pregnancy!

7.2 Standard range

2 to 400 HDU / mL

7.3 Sample volume

50 µL serum or EDTA-plasma

7.4 Reproducibility

Intraassay: < 15%
Interassay: < 20%

7.5 Incubation time

over night, 150 min

7.6 Storage of the Kit

Reagents should be stored at 2 - 8°C; shipment will be performed at ambient temperature

8 TECHNICAL HINTS

- To avoid cross-contamination, change pipette tips between addition of standards, control and samples. Also use separate reservoirs for each reagent!
- Do not mix up stoppers of different standards and samples
- Do not use reagents beyond expiration date
- Do not expose reagents to direct sunlight
- Do not mix or substitute reagents with those from other lots or sources
- Substrate solution must be colorless before use

9 PRECAUTIONS

- Do not pipette by mouth
- Do not eat, drink, smoke or apply cosmetics where reagents are used
- All liquid reagents contain 0.01%Proclin 300 as preservative. Proclin 300 is not toxic in concentrations used in this kit

10 FAQS

Is it possible to perform the assay without Multipette?

Multichannel pipette and reagent reservoirs can be used alternatively. Avoid cross-contamination by using specific reservoirs for every reagent.

How important is accuracy of the incubation temperature ?

To ensure reproducibility incubation temperature must be precise. 3°C difference can reduce signals already up to 50%!

Can I use different sealing tapes than the enclosed ones ?

No. To assure tight sealing of the plate kit enclosed tape must be used.

What are the critical points of the assay performance?

- All reagents must have room temperature
- After acylation of wells the solution must be mixed thoroughly
- Sealing of the plate for over night incubation must be made accurately to avoid drying
- There is a definite amount of histamine lyophilized into the incubation plate, therefore it is not feasible to manipulate well volume in other ways than described

What has to be considered at the standard dissolving step?

During the 15 minutes, but particularly before use of the standards the vials must be vortexed thoroughly.

What can I do if wells were dried up after over night incubation?

These wells cannot be used for a reasonable HDU calculation.

Is if normal that wells appear turbid after acylation step?
Yes. After 30 min the wells should become clear, if not mix solution with pipette.

Where should I store the Kit reagents after use?
Antibody solution and standards may be frozen twice at -20°C after use. Avoid repeated Freeze-thaw cycles.
All other Kit reagents have to be stored at 2 -8°C. Storage of resolved acylation reagent is not possible.

What has to be considered at the transfer step?
Before transfer content of incubation plate wells should be mixed with a pipette. Transfer the used strips quickly and without interruption.

Is it possible to use an automatically washer for the washing steps?
Yes.

What can I do if wells were not homogen after stopping of substrate reaction?
Shake plate shortly to mix Stop solution with TMB.

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..