Journal of Pharmaceutical and Biomedical Analysis 118 (2016) 307–314

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# Comparison of two enzymatic immunoassays, high resolution mass spectrometry method and radioimmunoassay for the quantification of human plasma histamine

Caroline Poli[a,b,c,d,e,1], Mathieu Laurichesse[f,g,1], Octavie Rostan[f], Delphine Rossille[h], Pascale Jeannin[a,b,c,d,e], Martine Drouet[i], Gilles Renier[e], Alain Chevailler[a,b,c,d,e], Karin Tarte[d,f,j,k], Claude Bendavid[g,j,l], Céline Beauvillain[a,b,c,d,e,**,1], Patricia Amé-Thomas[d,f,j,k,*,1]

[a] Université d'Angers, Faculté de Médecine, 49100 Angers, France
[b] INSERM, Unit 892, 49100 Angers, France
[c] CNRS, Unit 6299, Angers, France
[d] LabEx IGO "ImmunoGraft Oncology", France
[e] CHU Angers, Laboratoire d'Immunologie et d'Allergologie, Pôle Biologie, 49100 Angers, France
[f] CHU Rennes, Laboratoire d'Immunologie, Thérapie Cellulaire et Hématopoïèse, Pôle Biologie, 35033 Rennes, France
[g] CHU Rennes, Laboratoire de Biochimie, Pôle Biologie, 35033 Rennes, France
[h] CHU Rennes, Laboratoire d'Hématologie, Pôle Biologie, 35033 Rennes, France
[i] CHU Angers, Unité d'Allergologie générale, Département de Pneumologie, 49100 Angers, France
[j] Université de Rennes 1, Faculté de Médecine, 35043 Rennes, France
[k] INSERM Unité Mixte de Recherche U917, 35043 Rennes, France
[l] INSERM, Unité Mixte de Recherche U991, 35043 Rennes, France

ARTICLE INFO

*Article history:*
Received 28 September 2015
Received in revised form 28 October 2015
Accepted 1 November 2015
Available online 5 November 2015

*Keywords:*
Enzymatic immunoassay
Histamine
Mass spectrometry
Radioimmunoassay

ABSTRACT

Histamine (HA) is one of the main immediate mediators involved in allergic reactions. HA plasma concentration is well correlated with the severity of vascular and respiratory signs of anaphylaxis. Consequently, plasma quantification of HA is useful to comfort the diagnosis of anaphylaxis. Currently, radioimmunoassay (RIA) is the gold standard method to quantify HA due to its high sensitivity, but it is time consuming, implicates specific formations and cautions for technicians, and produces hazardous radioactive wastes. The aim of this study was to compare two enzymatic immunoassays (EIA) and one in-house liquid chromatography high-resolution mass spectrometry method (LC–HRMS) with the gold standard method for HA quantification in plasma samples of patients suspected of anaphylaxis reactions. Ninety-two plasma samples were tested with the 4 methods (RIA, 2 EIA and LC–HRMS) for HA quantification. Fifty-eight samples displayed HA concentrations above the positive cut-off of 10 nM evaluated by RIA, including 18 highly positive samples (>100 nM). This study shows that Immunotech® EIA and LC–HRMS concentrations were highly correlated with RIA values, in particular for samples with a HA concentration around the positive cut-off. In our hands, plasma concentrations obtained with the Demeditec Diagnostics® EIA correlated less with results obtained by RIA, and an underestimation of plasma HA levels led to a lack of sensitivity. In conclusion, this study demonstrates that Immunotech® EIA and LC–HRMS method could be used instead of RIA to assess plasma HA in human diagnostic use.



*Abbreviations:* EIA, enzymatic immunoassay; HA, histamine; LC–HRMS, liquid chromatography high resolution mass spectrometry; RIA, radioimmunoassay.

* Corresponding author at: Laboratoire d'Immunologie, Thérapie Cellulaire et Hématopoïèse Pôle Biologie—Hôpital Pontchaillou 2 Avenue du Pr Léon Bernard, 35033 RENNES Cedex, France.
** Corresponding author at: Laboratoire d'Immunologie et d'Allergologie Institut de Biologie en Santé (PBH)—CHU d'Angers 4, Rue Larrey, 49933 ANGERS Cedex 09, France.
*E-mail addresses:* celine.beauvillain@chu-angers.fr (C. Beauvillain), patricia.ame-thomas@chu-rennes.fr (P. Amé-Thomas).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jpba.2015.11.001
0731-7085/

## 1. Introduction

Anaphylaxis is a potentially lethal systemic type I hypersensitivity reaction [1]. This life-threatening emergency can lead very quickly to hypovolemia shock and cardiorespiratory arrest. In western countries, anaphylaxis incidence is estimated to be comprised between 4 and 50/100000 inhab/year with a prevalence of 0.05–2%. Emergency admission registers highlight a two fold increase of anaphylaxis prevalence during the last decades [2].

After a first exposure to an allergen leading to high-affinity IgE synthesis by long-lived plasma cells, these IgE are predominantly found linked to the high-affinity IgE receptor (Fc$\epsilon$RI) at the cell surface of mast cells and basophils. Following a second exposure to the same allergen, the cross-linking of the Fc$\epsilon$RI leads to cell activation and the release within a few minutes of a huge amount of histamine (HA) contained in cell granules ($10^{-5}$–$10^{-3}$ mol/L) [3,4], as well as other preformed proinflammatory mediators [5]. HA or 2-(1H-imidazol-4-yl) ethanamine is synthesized from L-histidine in the Golgi apparatus by the histidine decarboxylase [6]. Several cell types can synthetize HA in humans [7],but mast cells and basophils distinguish themselves by their ability to store HA in their granules, enabling a huge acute release of HA upon IgE cross-linking.

The anaphylaxis diagnosis is usually retained on a body of evidence. Suspicion is made primarily on clinical signs [8–10]. Currently, two biological parameters are commonly used to strengthen the diagnosis of anaphylaxis: serum tryptase and plasma HA. HA quantification is useful to confirm that clinical signs result from the degranulation of mast cells and/or basophils [11]. Physiologically, basal plasma HA amount is very low (<10 nM) [8] but can increase very quickly and exponentially after mast cell or basophil degranulation. Nevertheless, the HA protein half-life is extremely short [12]. Consequently, strict procedures of sampling for HA quantification are required: blood collection must be conducted within 15–60 min after the beginning of the symptoms and plasma isolation has to be performed very quickly before conservation [13]. The production of highly specific monoclonal antibodies targeting HA is extremely challenging due to the nature of this small biogenic amine (M = 111 g mol$^{-1}$). The former quantification assays were based on the covalently binding of HA to a carrier protein on imidazole core or on $NH_2$ end; nevertheless this chemical process was difficult to apply on plasma samples. Currently, classical HA quantification assays used for *in vitro* diagnosis (IVD) and based either on radioimmunoassay (RIA) [14] or enzymatic immunoassay (EIA) methods include an acylation step with NOH succinimide ester succinyl glycinamide, which turns HA into a bigger and more stable molecule that becomes easily detectable. Acylated plasma HA competes with exogenous acylated HA conjugated with a radioelement or an enzyme to bind a highly specific monoclonal antibody [15]. The current gold standard for HA quantification is RIA, it has the advantage of being highly sensitive [14], but requires the use of radioactive reagents implying the formation and the follow up of the technicians, as well as the dangerous and expensive waste management. More recently, two EIA kits for IVD use, both avoiding the use of radioelements, have been commercialized by Immunotech® and Demeditec Diagnostics®. Finally, some methods using mass spectrometry have been developed to quantify plasma HA [16,17]. In particular, our team developed an in-house plasma HA quantification assay for diagnostic use based on high Resolution Accurate Mass LC-MS (LC–HRMS) technology [18]. The LC–HRMS method is known to be highly sensitive and specific, averts radioactivity use, and avoids the potential detection of the metabolites of the targeted protein.

In this study, HA concentration was assessed in human plasma collected from patients with suspected anaphylactic reaction using three different methods: the gold standard RIA, the two commercialized EIA kits, and the in-house LC–HRMS-based method. This comparison reveals that Immunotech® EIA and LC–HRMS are both sensitive enough methods to be considered as relevant non-radioactive alternative methods to RIA.

## 2. Materials and methods

### 2.1. Plasma samples

Plasmas collected between February 2010 and February 2014 in routine clinical practice by laboratories of Angers and Rennes University Hospitals from patients following a suspected anaphylaxis reaction were tested for HA quantification. The Ethical Review Board in Angers approved this study (2015/40). Peripheral blood was collected by venipuncture in EDTA tubes, and plasma was separated by centrifugation at 3000 *g* for 10 min. Thereafter, plasma was aspirated gently while respecting a safety margin of 1 cm from the buffy coat in order to avoid contamination by white blood cells, aliquoted and immediately frozen at −20 °C.

### 2.2. Radioimmunoassay

HA quantification by RIA was performed following manufacturer's instructions (Immunotech, Marseille, France). Briefly, immediately after thawing, HA contained in plasma samples was acylated. Then, plasma samples were incubated overnight within monoclonal antibody (mAb)-coated tubes in presence of a $^{125}$I-labeled acylated HA internal tracer. Following incubation, the contents of the tubes were aspirated and bound radioactivity was measured using a Cobra 2 auto gamma counter (Packard instrument, Meriden, CT, USA). Limits of quantification given by the manufacturer were 0.2–100 nM. The limit for pathological values is 10 nM.

### 2.3. Enzymatic immunoassays

HA quantification by EIA was performed following manufacturers' recommendations (Immunotech® and Demeditec Diagnostics®, Kiel, Germany). For both kits, samples were manipulated in polypropylene tubes and tested in duplicate.

For Immunotech® EIA, HA was acylated by a first vortex step with an acylation reagent. Acylated control, standard and plasma solutions were further incubated in mAb-coated wells in presence of alkaline phosphatase acylated HA for 2 h at 2–8 °C under shaking (350 rpm). After incubation, wells were rinsed with a microtiter plate washer in order to remove non-bound components. The remaining enzymatic activity was then measured at 405 nm after the addition of a chromogenic substrate. A quadratic polynomial equation was used to define the standard curve; the correlation coefficient r was always >0.993 [0.987–0.999], and the median control values were 5.561 [95% CI 5.459–5.660] nM for theoretical value between 5.00 and 7.40 nM. Standard curve included dilutions from 1.00 to 100 nM of HA, as recommended. Limits of quantification given by the manufacturer were 0.50–100 nM.

For Demeditec Diagnostics® EIA, HA was acylated by incubation with an acylating reagent for 45 min at room temperature (RT) on a shaker (600 rpm) and further competed with plastic-coated HA for a precise amount of HA antibody binding sites added into the well. Equilibrium of the system was reached after 3 h at RT (20–25 °C) on a shaker (approximately 600 rpm). Then, free antibody and plasma HA-antibody complexes were removed by washing. The antibodies conjugated with plastic-coated HA were detected by a peroxidase-conjugated anti-rabbit IgG using tetramethylbenzidine as substrate. The reaction was monitored at 450 nm. A quadratic polynomial equation was used to define the standard curve, included standard concentrations between 4.5