Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms

Lindström, Mikael

2018-07


http://hdl.handle.net/10138/300392
https://doi.org/10.1016/j.cca.2018.03.030

Downloaded from Helda, University of Helsinki institutional repository.
This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.
Please cite the original version.
Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms: A pilot study

Mikael Lindström, Niina Tohmola, Risto Renkonen, Esa Hämäläinen, Camilla Schalin-Jäntti, Outi Itkonen

PII: S0009-8981(18)30146-3
DOI: doi:10.1016/j.cca.2018.03.030
Reference: CCA 15121
To appear in: Clinica Chimica Acta
Received date: 16 January 2018
Revised date: 22 March 2018
Accepted date: 24 March 2018

Please cite this article as: Mikael Lindström, Niina Tohmola, Risto Renkonen, Esa Hämäläinen, Camilla Schalin-Jäntti, Outi Itkonen, Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms: A pilot study. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Cca(2018), doi:10.1016/j.cca.2018.03.030

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms: a pilot study

Mikael Lindström a*, Niina Tohmola a, Risto Renkonen a,b, Esa Hämäläinen a,c, Camilla Schalin-Jäntti d, Outi Itkonen a

a HUSLAB, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
b Haartman Institute, University of Helsinki, Helsinki, Finland
c Department of Clinical Chemistry, University of Helsinki, Helsinki, Finland
d Endocrinology, Abdominal Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

*Corresponding author at: HUSLAB, P.O. Box 720, 00029 Helsinki University Hospital, Finland.
Email: mikael.lindstrom@hus.fi
Abstract

Background
Serotonin (5-hydroxytryptamine) is a mediator of gastrointestinal smooth muscle contraction, and is secreted by neuroendocrine neoplasms (NENs). We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay for serum serotonin to be used in NEN diagnostics and follow-up.

Methods
We used serum samples from healthy volunteers (n=31) and patients suspected or monitored for NEN (n=98). Serotonin-D_4 internal standard was added to samples before solid phase extraction (SPE) and quantification by LC-MS/MS. The effects of sample handling and preparation on serotonin stability were studied. Finally, we established a provisional reference range for serum serotonin and compared our assay with serum 5-hydroxyindoleacetic acid (5-HIAA) for detection of NENs.

Results
Our assay is sensitive and has a wide linear range (10-10000 nmol/l). Serum serotonin is stable for 7 days at room temperature and for 3 months at -20 °C. Sampling temperature is not critical. Normal range for serum serotonin was 270-1490 nmol/l. We found that serum serotonin and 5-HIAA performed equally well as diagnostic tests for NENs.

Conclusions
Our LC-MS/MS assay for serum serotonin is well suited for clinical research and patient diagnostics. Our results confirm that it can complement 5-HIAA in diagnosis of NENs.

Keywords: Serotonin, 5-HIAA, NEN, Neuroendocrine neoplasm, LC-MS/MS
Abbreviations: NEN, neuroendocrine neoplasm; LC-MS/MS, liquid chromatography tandem mass spectrometry; 5-HIAA, 5-hydroxyindoleacetic acid; SPE, solid phase extraction
Introduction
Serotonin (5-hydroxytryptamine) is a monoamine neurotransmitter synthesized from the amino acid tryptophan. Most of total body serotonin is found in the gastrointestinal (GI) tract, where it modulates the functions of the bowel by controlling smooth muscle contraction. It also acts as a cardiovascular vasoconstriction mediator and in the brain as a neurotransmitter. Any irregularities in the serotonergic system may thus contribute to various clinical conditions such as bowel motility disorders, cardiac abnormalities, and negative effects on the mood and memory regulation [1].

Neuroendocrine neoplasms (NENs), which originate from GI tract enterochromaffin cells, may produce large amounts of serotonin. Tumors of the fore- and midgut produce predominantly serotonin, while production is usually minimal in tumors of the hindgut. The incidence of NEN is relatively low, 5:100000, but diagnoses are becoming more common [2], primarily due to improvements in early detection of the disease. NENs occur in all age groups, but are the most common in patients aged 60-70 years. Traditionally, the diagnosis of these tumors is based on symptoms, detection of serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) [3] and chromogranin A (CgA) [4], and imaging results. Some studies have proposed platelet, plasma or urinary serotonin assays to complement these [5,6] due to their high sensitivity in detecting especially serotonin producing midgut tumors.

Previous assays for serotonin are immunoassays or based on liquid chromatography with fluorometric [7], electrochemical (EC) [8] or mass spectrometric detection [9,10]. Many previously published methods employ platelet poor plasma, where serotonin concentration is low and sample handling may be laborious and prone to errors [6,8,11-13]. As platelets readily store serotonin via an active uptake process, serotonin concentration in regular serum is significantly higher and sample handling and preparation more straightforward. Plasma serotonin concentration has also been found to be affected by dietary intake of serotonin-rich foods while serum serotonin is not [14].

We have previously used a serum 5-HIAA assay for NEN diagnostics [3]. To complement our earlier work, we developed a new LC-MS/MS assay for serum serotonin and evaluated the discriminating power of serotonin as a NEN marker as compared to our previous serum 5-HIAA test. Some diagnostic laboratories [15] suggest cold sampling procedures for serotonin. Therefore, we also wanted to investigate, whether cold sampling procedure is necessary and studied preanalytical factors such as sample stability and sampling device.

2. Materials and methods
2.1. Reagents and materials
Stock solutions of 10 µmol/l (1.76 mg/l) serotonin (Sigma-Aldrich Co) in 1% acetic acid (174 mmol/l) and deuterium labeled serotonin-D4 internal standard (IS) (Medical isotopes Inc. Pelham, NH, USA) in water were prepared. Serotonin calibrators in water (0.06-10000 nmol/l) and IS working solution (1000 nmol/l) in 20 mmol/l ammonium acetate were freshly prepared from the stock solutions. Sample pretreatment was performed employing 96-well Oasis WCX μElution plates (Waters, Milford, MA, USA). MS-grade methanol, acetonitrile (ACN), formic acid, acetic acid and ammonium formate were from Fluka (Sigma-Aldrich Co.). All reagents were of the highest analytical grade.

2.2. Samples and patients
Serum and plasma samples from apparently healthy volunteers were obtained in May 2016 from our laboratory staff (30 women and 6 men) with a median age of 48 yrs (range 27-65 yrs). Informed consent was obtained from all individuals. The samples were kept at -20 °C until analysis unless otherwise stated. Patient samples (50 women, 48 men) were from individuals suspected or followed for active NEN (assessed by clinical examination, other blood tests, including CgA and 5-HIAA and radiological studies) at the Endocrine Department and from whom serum samples were drawn and analyzed for 5-HIAA during 2016 as part of their diagnostic work-up as previously described [3] (Table 1). Most patients (ca. 70%) had been diagnosed at some point with a tumor of the ileum, the rest were divided among other midgut tumors (2%), foregut tumors (15%), non-GI tumors (4%) and unconfirmed tumor (9%). This study was approved by the ethical committee of Helsinki University Central Hospital, Finland.

2.3. Sample and calibrator preparation
Samples and calibrators (100 µl) were pipetted into the wells of a 96-well plate along with 100 µl of IS working solution. A sample volume of 200 µl was used for platelet poor plasma (PPP). WCX μElution plate was wetted with 200 µl of methanol and 200 µl of water. Samples and standards including the IS were transferred into the μElution wells followed by washing with 200 µl of 20 mmol/l ammonium acetate and 200 µl of methanol. Serotonin was then eluted with 100 µl of methanol/formic acid/ water (30/5/65, v/v) and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

2.4. LC–MS/MS
The system comprised an Agilent 1200 liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a 4000 QTRAP triple quadrupole mass spectrometer (AB Sciex, Toronto,
Canada) equipped with a Turbo-V electrospray ion (ESI) source. For separation, Atlantis HILIC Silica 50 x 2.10 mm 3 µm column (Waters) was used and operated at 40 °C. Elution buffers were ACN (A) and 90 mmol/l ammonium formate, pH 4 (B). The column was kept at 5% B for 0.5 min, ramped from 5% to 50% B in 0.5 min, from 50% to 5% B in 0.6 min and kept at 5% B for 6.4 min. The flow rate was 300 µl/min. For MS/MS detection transitions m/z 177 → 115 and m/z 181 → 164.2 for serotonin and the IS were followed, respectively. The ion source was kept at 5000 V and at 600 °C in positive ion mode. Settings for the curtain, nebulizer and heater gases were 20, 60 and 50 l/min, while the collision gas setting was at 9. Data was collected and processed by the Analyst software (Version 1.6.2, AB Sciex).

2.5. Analytical validation of the method
The linearity was determined by preparing and analyzing 15 calibrator dilutions (0.625-10000 nmol/l) on three different days. The calibration curve was derived using 1/x² weighted linear least-squares regression. Relative error (RE%) and the coefficient of variation (CV%) were calculated to assess accuracy and precision, respectively. The limit of detection (LOD) was determined as the lowest concentration with a signal to noise ratio of 3. Limit of quantification (LOQ) and linear range was determined as the lowest concentration and range, respectively, that could be measured with RE and CV <20%. For quality assurance (QA), we employed Clinchek level 1 and 2 Plasma Controls for biogenic amines (RECIPE, Munich, Germany) containing 590 and 1700 nmol/l serotonin, respectively. Intra- and inter-assay variation were calculated from the QA sample results in a single batch (n=12) and on 14 separate days, respectively. Recovery of added serotonin was determined using three serum samples (endogenous serotonin concentration 207-803 nmol/l) with and without serotonin spike of 250 and 1000 nmol/l. Matrix effect was studied by extracting two serum samples in triplicate, spiking with 250 nmol/l of serotonin after sample extraction and comparing with spiked elution buffer. Effect (attenuation or enhancement) on the measured signal was calculated from the peak areas.

2.6. Preanalytical validation
We studied sample stability using fresh serum samples (n=15) from healthy individuals aliquoted at room temperature, +4 °C and -20 °C. The non-frozen samples were analyzed on days 0, 1, 2, 3, 4 and 7. The frozen samples were analyzed on days 0, 7, 21, 28, 42, 56, 84 and 180. The effect of repeated freezing and thawing from the same aliquot was studied on the same days. Samples with <20% change from initial concentration were considered stable.
The effect of sampling device was studied with blood samples collected from 31 healthy volunteers. Samples were drawn into plain serum tubes, serum activator tubes (CAT), serum gel tubes (SST™ II Advance, all from BD Vacutainer, Plymouth, UK) and lithium-heparin tubes (Venosafe 60 USP U Lithium Heparin, Terumo, Leuvon, Belgium). Separation of serum or plasma was performed as suggested by the respective manufacturer and analyzed immediately. Suggested minimum clotting time for serum was 30 min (actual 60 min). Plasma samples were separated in 15 min. Delayed (up to 6 hrs) centrifugation after sampling (n=6) on serotonin concentration was studied using plain serum tubes. The effect of sampling temperature was tested with duplicate samples (n=11) kept either at room temperature or +4 °C through the entire sampling and preanalytical process.

For preparation of platelet poor plasma (PPP, n=12), samples from healthy individuals were drawn into citrate plasma tubes (BD Vacutainer). After 15 minutes, the samples were centrifuged at 3000 g for 10 min, the top 2/3 of plasma collected and re-centrifuged.

2.7. Serum 5-HIAA assay
Serum 5-HIAA was analyzed as previously described [3]. Briefly, serum samples were extracted using Oasis WAX μElution plates by washing with 2% formic acid and 60% ACN and then eluting with the LC buffer (97% A, 3% B, same eluents used as in here). The MS transitions were m/z 192.1 → 146 for 5-HIAA and m/z 194 → 147.9 for IS.

2.8. Statistical methods
We used Analyse-it for Microsoft Excel 2016 (Ver. 4, Analyse-it software Ltd, http://www.analyse-it.com) to establish a provisional reference range for serum serotonin by a parametric method, to run receiver operating characteristic (ROC) analysis, Deming regression and paired t-tests. A p-value <0.05 was considered statistically significant.

3. Results
3.1. Analytical validation of the serotonin assay
The retention time of serotonin and IS was 3.27 min (Figure 1). The method was linear over the concentration range 10-10000 nmol/l, the LOD was 2.5 nmol/l and LOQ 10 nmol/l. Routinely, we used 10-5000 nmol/l calibrators. The intra-assay CVs were 3.5% and 5.4% and the interassay CVs 5.2% and 7.8% at 590 and 1700 nmol/l serotonin, respectively. The mean recoveries of serotonin (250 and 1000 nmol/l) spiked into serum samples (n=3) containing 270-803 nmol/l
endogenous serotonin were 102% (range 95-115%) and 101% (range 97-109%), respectively. Sample matrix suppressed the measured serotonin and IS signal by 26%.

3.2. Preanalytical validation
There was no difference in serotonin concentrations in samples drawn into serum activator (mean 843, range 407-2060 nmol/l) or gel tubes (mean 855, range 398-2060 nmol/l). However, serotonin concentrations were lower in samples drawn into plain serum tubes (mean 707, range 262-1550 nmol/l, p=0.0001), into lithium-heparin tubes (mean 48, range 33-89 nmol/l, p<0.0001) and in PPP (mean 11, range 5-30 nmol/l, p=0.0005) (Figure 2). In this study we used samples drawn into plain serum tubes unless otherwise stated.

The measured mean serum serotonin concentration was 15% (797 nmol/l, p=0.0327) and 20% (767 nmol/l, p=0.0187) lower than the initial 958 nmol/l after a delay of 4 hrs and 6 hrs before centrifugation, respectively. There was no difference observed between 1 and 2 hrs delay time. Cooled sampling during the preanalytical phase was not critical (p=0.8855, Figure 3).

Serum serotonin was stable for at least seven days at room temperature (mean 631-711 nmol/l) and +4 °C (mean 645-713 nmol/l), for 84 days at -20 °C (mean 629-711 nmol/l) and for seven freeze-thaw cycles (mean 621-711 nmol/l) (Figure 4).

3.3. Serum serotonin and 5-HIAA concentrations
Mean serum serotonin and 5-HIAA concentrations in healthy individuals was 632 nmol/l (range 262-1550 nmol/l) and 67 nmol/l (range 30-140 nmol/l), respectively. The upper reference limit of our serum 5-HIAA assay is 123 nmol/l [3]. We suggest a provisional reference range of 270-1490 nmol/l for serum serotonin (95% confidence intervals 210-342 nmol/l and 1170-1900 nmol/l for lower and upper limits, respectively) (Figure 5). In our study, genders did not differ significantly (p=0.661) in serum serotonin content.

In patient samples, serum serotonin concentration was 42-13200 nmol/l (mean 1833 nmol/l) and that of 5-HIAA was 23-3170 nmol/l (mean 366 nmol/l). On average, serum serotonin concentration was 5-fold compared to serum 5-HIAA. Serum serotonin and 5-HIAA concentrations of all samples correlated according to y (serotonin, nmol/l) = 398.4 + 3.919 x (5-HIAA, nmol/l) by Deming regression (Sy|x=2663, n=128). Four of 39 active NEN patients had no elevation in either analyte concentration, among them one patient had a non-GI tumor, one with pancreatic tumor and two with ileum tumors. Two patients with midgut tumors (ileum) had elevated serotonin only. Finally, six patients had elevated 5-HIAA but normal serotonin concentration. Of these, four had tumors in pancreas, one in liver and one in ileum. In the
remaining NEN samples (n=27) the concentration of both analytes was elevated above the upper reference limit. In ROC analysis, the areas under curve (AUC) for serotonin and 5-HIAA were 0.91 and 0.94, respectively (Table 2). With cut-off values of 1490 nmol/l for serotonin and 123 nmol/l for 5-HIAA, we achieved sensitivity of 75% and 85%, and specificity of 92% and 85%, respectively.

4. Discussion
There are few extensively validated LC-MS/MS-based serotonin assays in literature. The LOQ for our new serotonin LC-MS/MS assay was 10 nmol/l and the linear range is 10-10000 nmol/l. A better sensitivity can be achieved by using 200 µl sample volume instead of 100 µl as we used for PPP samples with <10 nmol/l serotonin. Previously described serotonin assays have LOQ of 1-50 nmol/l and linear range of 3.5-5000 nmol/l [9,10,16-18]. Our assay compares favorably with these with inter- and intra-assay CVs of <10 %.

Methods based on immunoassays and HPLC paired with fluorescence, UV or electrochemical detection may be laborious and suffer from interference from related compounds present in samples. MS based detection has excellent specificity and sensitivity for clinical applications. Sample treatment of our assay relies on SPE, while others have reported a simple precipitation step sufficient [17]. Time-consuming derivatization procedures like that in [19] are not required. In this study, serum serotonin was stable at -20 °C for 84 days and during 5 freeze-thaw cycles. Serotonin was stable for one week when stored at +4 °C and at room temperature, provided that the samples had been initially centrifuged within two hours. These results confirm those of previous reports [10,20]. Therefore, serum samples can be handled and transferred at room temperature, which is an advantage for today’s centralized clinical laboratories.

We found that plain serum tubes yielded slightly lower serotonin concentrations when compared to gel or clotting activator tubes. Clotting is known to release serotonin from platelets [21]. This finding suggests that this process is more efficient in gel and in clotting activator tubes than in plain serum tubes. Plasma samples drawn to lithium-heparin tubes produced markedly lower results, while PPP samples drawn sodium citrate tubes were the lowest in serotonin concentration, due to smaller platelet amount in PPP. For serum assay, any of the tested sampling devices can be used, but the reference values need to be established for each sampling device. We used samples drawn into plain serum tubes in this study. Previously reported LC-MS/MS methods have widely used platelet rich plasma (PRP), PPP or urine as the sample matrix. When preparing PRP or PPP for serotonin analysis, careful sample handling is required, as serotonin easily leaks from platelets into plasma. Reported mean PPP
serotonin concentrations vary widely between studies, i.e. from 0.6 to 180 nmol/l [13], although the few MS-based assays report levels of 1-5 nmol/l. This discrepancy is probably due to differences in plasma treatment procedures, resulting in varying amounts of platelets present, as well as different analytical techniques. Our PPP serotonin results (mean 10.7, range 5.3-29.9 nmol/l, n=12) are similar to those reported by de Jong et. al.[9] using an LC-MS/MS assay (mean 4.6 nmol/l, range 2-15 nmol/l, n=22). Our samples drawn into lithium-heparin tubes produced higher results as expected. These samples were not doubly centrifuged and thus they were likely to contain platelets.

We found that serum serotonin concentration decreased upon delayed (4 and 6 hrs) sample centrifugation (mean concentration 797 and 767 nmol/l) compared to standard separation (mean 957 nmol/l, p=<0.033, n=11). In the body, serotonin is degraded by the enzyme monoamine oxidase (MAO [6]) and in circulation the half-life of serotonin is 1.3 hrs [22]. MAO activity may cause serotonin degradation also in vitro, but in this study the mechanism was not elucidated. There was no difference in serotonin concentrations between cold sampling and treatment (mean 1018 nmol/l) as compared to handling at room temperature (mean 1004 nmol/l, p=0.9157). Taken together, our limited study suggests that sampling temperature is not critical, but a delay of 4 hrs or more between sampling and sample centrifugation may affect serotonin concentration in the sample.

A previous study suggest that gender does not influence circulating levels of serotonin [23]. Likewise, we found no statistical difference between the genders. Therefore, we established an upper 95% provisional reference limit of 1490 nmol/l for serum serotonin in adults (combined gender). This result is in line with other studies. Korse et al. [10] suggest an upper limit of 1356 nmol/l using LC-MS/MS assay detection, while methods based on fluorescence or EC report concentrations of ca. 1200-2000 nmol/l (given as 5.1-7.8 nmol/10^9 platelets) [7,11,24,25].

Comparison of serotonin concentrations in serum samples from healthy volunteers and NEN patients either in remission or with an active disease showed a clear difference between the groups. Patients with confirmed active NEN had significantly elevated serum serotonin levels, while in samples from patients in remission it differed only slightly from that in healthy volunteers. When we compared serum serotonin and serum 5-HIAA assay by ROC analysis (Table 2), both methods discriminated equally well between healthy subjects and patients with active disease with AUCs 0.91 and 0.94, respectively. Our findings are in accordance with those of Meijer et al. (19). In their study, platelet serotonin (AUC 0.89) was slightly better at discriminating carcinoid patients from the healthy as compared to urinary serotonin (AUC 0.84) or urinary 5-HIAA (AUC 0.79). In our study, serum serotonin was most sensitive for detecting
NENs of the midgut, while 5-HIAA was slightly better as a foregut tumor marker. This is also in line with other studies [5,26], and is linked to the different serotonin secretion rates between tumors of the fore- and midgut, as well as their varying enzymatic synthesis rate from tryptophan to serotonin.

In conclusion, our new LC-MS/MS assay for serum serotonin suits well for routine clinical laboratories for NEN diagnostic and for research purposes. The linear range covers a wide physiological range including both normal subjects and NEN patients’ serotonin concentrations. Serum samples are stable for several days at room temperature or months when frozen. The established provisional cut-off-value of 1490 nmol/l discriminates well between healthy individuals and patients with active NENs. Further studies to confirm the clinical utility of serum serotonin in NEN diagnostics are warranted.

Funding
C Schalin-Jäntti received grants from Helsinki University Hospital Research Funds (TYH2016129 and TYH2017138), Finska Läkaresällskapet and Ipsen Nordic

Disclosures
Declarations of interest: none.

Acknowledgements
The authors thank the HUSLAB Endocrinology and Metabolism Laboratory staff for sample donation and for their expert technical assistance.
References


Figure 1. Chromatograms of a serotonin calibrator (A), of a serum sample from a healthy individual (B) and from a NET patient (C) with 500, 605 and 8100 nmol/l of serotonin, respectively.

Figure 2. Distribution of serotonin concentrations in samples drawn into plain serum (serum), serum activator (CAT), serum gel and plasma (Li-heparin) tubes from 31 healthy donors.

Figure 3. Effect of sample handling temperature on serotonin concentration on parallel samples (n=11).

Figure 4. Mean (±SD) serum serotonin stability of samples (n=15) drawn into plain serum tubes at -20 °C.

Figure 5. Serotonin (A) and 5-HIAA (B) concentrations in serum from healthy individuals (NoNEN, n=89) and from active NEN patients (n=39). The dotted line shows the respective upper reference limits of 1490 nmol/l and 123 nmol/l.
Table 1. Details of the NEN patients.

<table>
<thead>
<tr>
<th>Information</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63.8 (26-86)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>48/50</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>In remission</td>
<td>58</td>
</tr>
<tr>
<td>Time from diagnosis, years</td>
<td>4.2 (0-13)</td>
</tr>
<tr>
<td>Active NEN</td>
<td>39</td>
</tr>
<tr>
<td>Time from diagnosis, years</td>
<td>3.7 (0-12)</td>
</tr>
<tr>
<td><strong>Tumor location</strong></td>
<td></td>
</tr>
<tr>
<td>Foregut</td>
<td>14</td>
</tr>
<tr>
<td>Lungs / Liver / Pancreas</td>
<td>4/1/9</td>
</tr>
<tr>
<td>Midgut</td>
<td>70</td>
</tr>
<tr>
<td>Ileum / Appendix</td>
<td>68/2</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
</tr>
<tr>
<td>Adrenal gland / kidneys / thyroid gland</td>
<td>2/1/1</td>
</tr>
</tbody>
</table>
Table 2. AUC, sensitivity and specificity of serum serotonin and serum 5-HIAA assays. Cut-off values were 1490 nmol/l and 123 nmol/l for serum serotonin and 5-HIAA, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Serum serotonin</th>
<th>Serum 5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy/Remission/Active NEN (n)</td>
<td>31/58/39</td>
<td>31/58/39</td>
</tr>
<tr>
<td>AUC</td>
<td>0.91</td>
<td>0.94</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>92</td>
<td>85</td>
</tr>
<tr>
<td>Remission/Active NEN (n)</td>
<td>58/39</td>
<td>58/39</td>
</tr>
<tr>
<td>AUC</td>
<td>0.90</td>
<td>0.93</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>90</td>
<td>83</td>
</tr>
<tr>
<td>Healthy/Active NEN (n)</td>
<td>31/39</td>
<td>31/39</td>
</tr>
<tr>
<td>AUC</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>97</td>
<td>90</td>
</tr>
</tbody>
</table>
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5

A

B

p<0.0001

No NEN  Active NEN  No NEN  Active NEN

Concentration, nmol/l
Highlights

- A new LC-MS/MS assay for serum and plasma serotonin was developed
- Analytical performance was good and assay covered relevant clinical concentrations
- Sampling and stability were studied and found suitable for clinical laboratory
- Serum serotonin can be used to discriminate the healthy from the NEN patients