Anal Bioanal Chem
DOI 10.1007/s00216-011-5583-1

ORIGINAL PAPER

Evaluation of a novel ELISA for serotonin: urinary serotonin as a potential biomarker for depression

Mikaela I. Nichkova · Han Huisman · Paul M. Wynveen · David T. Marc · Kelly L. Olson · Gottfried H. Kellermann

Received: 19 September 2011 / Revised: 10 November 2011 / Accepted: 15 November 2011
© Springer-Verlag 2011

Abstract Depression is a common disorder with physical and psychological manifestations often associated with low serotonin. Since noninvasive diagnostic tools for depression are sparse, we evaluated the clinical utility of a novel ELISA for the measurement of serotonin in urine from depressed subjects and from subjects under antidepressant therapy. We developed a competitive ELISA for direct measurement of serotonin in derivatized urine samples. Assay performance was evaluated and applied to clinical samples. The analytical range of the assay was from 6.7 to 425 μg serotonin/g creatinine (Cr). The limit of quantification was 4.7 μg/g Cr. The average recovery for spiked urine samples was 104.4%. Average intra-assay variation was 4.4%, and inter-assay variation was <20%. The serotonin analysis was very specific. No significant interferences were observed for 44 structurally and nonstructurally related urinary substances. Very good correlation was observed between urinary serotonin levels measured by ELISA and liquid chromatography tandem mass spectrometry (LC-MS/MS; ELISA=1.16×LC-MS/MS−53.8; r=0.965; mean % bias=11%; n=18). Serotonin was stable in acidified urine for 30 days at room temperature and at −20 °C. The established reference range for serotonin was 54–366 μg/g Cr (n=64). Serotonin levels detected in depressed patients (87.53±4.89 μg/g Cr; n=60) were significantly lower (p<0.001) than in nondepressed subjects (153.38±7.99 μg/g Cr). Urinary excretion of serotonin in depressed individuals significantly increased after antidepressant treatment by 5-hydroxy-tryptophane and/or selective serotonin re-uptake inhibitor (p<0.01). The present ELISA provides a convenient and robust method for monitoring urinary serotonin. It is suitable to monitor serotonin imbalances and may be particularly helpful in evaluating antidepressant therapies.

Keywords Immunoassay · ELISA · Urine · 5-Hydroxytryptamine · Depression · Antidepressants

Electronic supplementary material The online version of this article (doi:10.1007/s00216-011-5583-1) contains supplementary material, which is available to authorized users.

M. I. Nichkova (✉) · H. Huisman · P. M. Wynveen
Department of Research and Development, Pharmasan Labs, Inc.,
373 280th Street,
Osceola, WI 54020, USA
e-mail: mikaela.nichkova@pharmasan.com

D. T. Marc · K. L. Olson · G. H. Kellermann
NeuroScience Inc.,
373 280th Street,
Osceola, WI 54020, USA

Introduction

Biogenic amines are structurally related to a group of organic compounds designated as catechols (e.g., dopamine, norepinephrine, and epinephrine) and indoleamines (e.g., serotonin) which have been researched for decades [1–3]. Most of the antidepressants (e.g., tricyclics and heterocyclics), monoamine oxidase inhibitors, and supplements (e.g., 5-hydroxy-tryptophan (5-HTP)) given by psychiatrists and other health care providers have one or more of the biogenic amines as their biological target.

Biogenic amines, such as serotonin (5-hydroxytryptamine) and norepinephrine, are critical molecules in pathways that function in mood and depressive expression [4, 5]. As such, a well-maintained metabolic pathway of serotonin is a prerequisite for proper functioning of the nervous system. Clinical observations of individuals with lesions or improper serotonin synthesis, have given rise to seminal findings of the brain–behavior influence of serotonin [6, 7]. A commonality in seemingly disparate conditions such as depression, anxiety and violent behavior, is the manifestation of brain serotonin deficiencies [8–10]. On the contrary, elevated levels of

Published online: 10 December 2011

serotonin in circulation of individuals with autism are one of the well-replicated findings in biological psychiatry [11, 12]. Thus, the influence serotonin has on brain–behavioral relationships in humans needs further investigation, aimed at optimizing approaches to improve health after treatment with nonpharmaceuticals and/or psychopharmaceuticals [5, 13–15].

The measurements of serotonin levels have a significant potential as a clinical tool and should logically be performed within neurons or in the extracellular space. The measurement of serotonin in cerebrospinal fluid (CSF) could provide, at best, the indication of the average serotonin concentration in all brain regions at one time point. The extreme low concentrations of serotonin in CSF make it unlikely that CSF serotonin will be of clinical utility in assessing central serotonergic function [16, 17]. For obvious reasons, CSF serotonin does not allow large scale screening and sampling at different time points. Urinary and plasma measurements of serotonin using liquid chromatography in combination with mass spectrometry, electrochemical or fluorometric detection [18, 19] have been used in medicine as a screening test for specific physiological conditions such as carcinoid tumors and liver cell regeneration [3, 20]. Other assays for serotonin include radioimmunoassay and ELISA which use chemical modifications of serotonin prior to analysis [21, 22]. Under normal conditions, urinary serotonin mainly reflects release from enterochromaffin cells in the gut, but also from the synthesis in the kidney, release from platelets and may include some release of the brain [1]. In neurological diseases where urinary, plasma and/or platelet serotonin is elevated, as in autism [10–12] and during selective serotonin re-uptake inhibitor (SSRI) intake (Ref. [15] and this paper), or is decreased in subjects with depression (this paper), the etiology of the serotonin imbalance is unknown.

We describe here the evaluation of an ELISA suitable for determination of urinary serotonin as a routine clinical assay. The test is useful in monitoring serotonin levels and for assessing the effectiveness of antidepressant treatment.

Materials and methods

Reagents

Unless otherwise stated, all reagents were of analytical grade and purchased from Sigma Chemicals (St. Louis, MO).

Polyclonal antibodies

Rabbit polyclonal serum was raised against serotonin conjugated with glutaric aldehyde to thyroglobuline as described in [23]. Specific polyclonal antibodies were isolated by serum depletion against carrier protein. The specificity of the serotonin antibody was evaluated in a standard ELISA to determine cross-reactivity at 50% displacement of structurally related compounds, precursors and/or metabolites of serotonin, such as 5-hydroxyindoleacetic acid, 5-HTP, 5-hydroxytryptophol, 5-methoxytryptamine, melatonin, *N*-acetylserotonin (*N*-Ac-5HT), tryptamine, and tryptophan. All tested compounds have cross-reactivity lower than 1.5%, except for *N*-Ac-5HT (8.4%).

Urine samples and study populations

Second void morning urine samples (spot samples) were collected in 5-mL tubes containing a filter disc impregnated with 250 μL of 3 N HCl as preservative. Samples were stored at −20 °C until analysis. To evaluate the reference range second void urine samples were obtained from 64 apparently healthy volunteers as determined by the results of the Hopkins Symptoms Checklist-90 (HSCL-90) [24–26]. Depressed subjects used in these clinical studies had depression as indicated by their medical history and/or International Classification of Diseases and Related Health Problems (ICD-9 codes) provided by their medical doctor (296.20–296.25, 296.30–296.35, 296.50–296.55, 300.4*x*, 309.0*x*, 309.28, and 311.*xx*). All subjects included in the clinical studies had no cancer as indicated by their medical history and/or ICD-9 codes. All individuals gave their informed consent. The studies were performed following a protocol set up by the internal clinical ethics committee.

Derivatization of standards, controls, and samples

Stock solutions for serotonin standards and controls were prepared in 150 mmol/L HCl. Urinary creatinine (Cr/100 dL) was determined by Urinary Creatinine Jaffe 2 Method on Roche Integra 400/880. To normalize for differences in urine excretion volumes, a volume of urine (40–300 μL) equivalent to 120 μg Cr was used in the test. Standards (120 μL), controls (120 μL), and samples were diluted up to 300 μL with 150 mmol/L HCl and neutralized with 60 μL of 1 mol/L $NaHCO_3$ containing 0.3% BSA and 0.1%Tween 20. In situ conjugation was performed with 120 μL of 267 mmol/L glutaric aldehyde. After 1-h incubation at 20 °C in the dark, the reaction was stopped with 120 μL Tris-quenching buffer (666 mmol/L Tris–HCl, pH 7.5; 344 mmol/L NaCl; 0.25% Tween 20; 0.5% BSA; 0.02% sodium azide). The derivatized standards and samples were incubated for 30 min at 20 °C in the dark and then applied to the ELISA.

Competitive ELISA

Similar procedures have been described for other biogenic amines [23, 27]. Briefly, microtiter plates were coated with BSA-serotonin in 50 mmol/L sodium carbonate-bicarbonate buffer pH 9.6. Derivatized serotonin standards, controls, and

samples (75 µL/well) were added to the coated plates followed by the serotonin-specific antibody (75 µL/well). After overnight incubation (32 °C, under shaking), a solution of secondary antibody (goat anti-rabbit IgG-alkaline phosphatase, 150 µL/well) was added and incubated for 60 min (20 °C, shaking). Finally, 150 µL/well of substrate was added and the absorbance was read at 405 nm on a Spectramax microplate reader (Molecular Devices Corp., Sunnyvale, CA). The competitive curves were analyzed with a four-parameter logistic equation using SoftmaxPro v5.4 (Molecular Devices Sunnyvale, CA). The concentration was calculated from the standard curve and expressed as micrograms serotonin per gram Cr.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc, San Diego, CA) and R Foundation for Statistical Computing, v2.11.0 (R Development Core Team, Vienna, Austria). Deming regression analysis and Bland–Altman approach were used to assess the correlation between ELISA and LC-MS/MS measurements. Linear regression and Pearson correlation coefficients were used for sample stability studies and comparison of serotonin levels in 24-h and spot urine. Parametric reference interval (95th percentile) was determined according to the recommendations of Clinical and Laboratory Standards Institute (CLSI), International Federation of Clinical Chemistry, and the International Union of Pure and Applied Chemistry (IUPAC) [28, 29]. The differences between serotonin levels in depressed and nondepressed individuals were examined using two-sample *t* test. A two-way ANOVA was used to compare baseline and retest serotonin levels within and between groups of depressed subjects under antidepressant therapy. *p* values of <0.05 were considered statistically significant.

Results

Linearity and detection limit

Assay linearity was investigated as suggested in CLSI Protocol EP6 [30]. A series of samples of known concentrations (assigned values) were created by sequential mixing of low and high concentration pools of urine. Linearity at specific concentrations were considered acceptable if the % difference between the predicted 1st- (linear) and 2nd- order regressed values (deviation %) was less than ±15% (see Fig. 1; Table S1 in the Electronic supplementary materials (ESM)). The analytical measurement range of the assay was determined to be

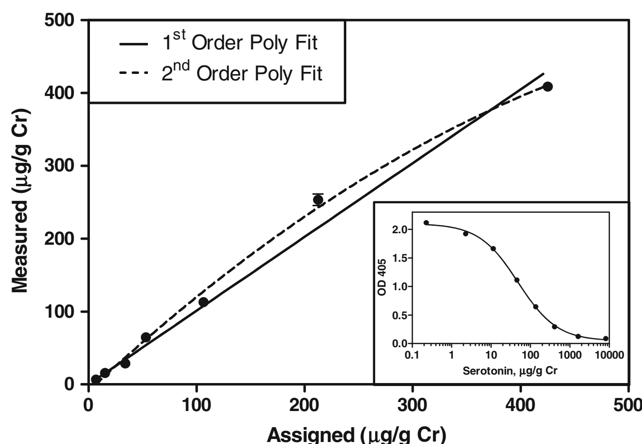


Fig. 1 Linearity (analytical measurement range). A series of urine samples with known concentrations of serotonin (assigned value) was created by sequential mixing of pools of urine with low and high concentration of serotonin. The predicted 1st- and 2nd-order regressed values (deviation %) were ±15%. The linear (analytical measurement) range was from 6.7 to 425 µg/g Cr. *Insert*, representative serotonin ELISA standard curve

from 6.7 to 425 µg/g Cr. Urine samples with highly elevated serotonin levels (>425 µg/g Cr) were measured accurately at dilutions up to 1:16 (for dilutional linearity see Table S2 in the ESM). Based on a 2 SD evaluation of the zero calibrator, the limit of detection of the ELISA (95% confidence) was determined to be 0.92 µg/g Cr. The lower limit of quantification (lowest concentration for which the 95% confidence interval (CI) of the CV% is less than 20%) was 4.7 µg/g Cr.

Imprecision

To assess reproducibility of the serotonin ELISA two urine samples (low and high level) and two in-house controls were analyzed in duplicate on a single plate over 18 days. Intra-assay variation (CV%) within plate was <10% with an average intra-assay variation for samples and controls of 4.4%. The inter-assay variation ($n=18$) was less than 20% (see Table S3 in the ESM).

Analytical recovery

The analytical recovery (accuracy) was determined by adding serotonin to urine samples with low endogenous amounts of serotonin. The test sample concentrations were between 6.7 and 8864.2 µg/g Cr. Each sample was analyzed (spiked and nonspiked) in duplicate and the percent recovery was calculated. The amount of serotonin recovered ranged from 84.7 to 122.3%, and the average recovery was 104.4% (see Table S4 in the ESM).

Analytical specificity and interferences

Interference studies were conducted according to CLSI Protocol EP7-A2 [31]. Clinically high concentrations of 44 potentially interfering substances (general urinary components, structurally similar urinary components, drugs and supplements) were spiked in urine samples with known levels of serotonin (60 and 383 μg/g Cr) and assayed along with nonspiked urine samples. The substances presented in Table 1 were found not to interfere at the concentrations indicated (<15% bias; upper 95% CI).

Comparison of ELISA with LC-MS/MS

The serotonin ELISA analysis of 18 urine samples was compared with LC-MS/MS performed by Mayo Medical Laboratories (Rochester, MN). Samples included in the study contained serotonin at concentrations ranging from within the reference interval to significantly increased. The results of the Deming regression analysis are shown in Fig. 2a (slope is 1.16; intercept is −53.8; r=0.965; N= 18). Although the serotonin concentrations obtained by the immunoassay showed a tendency to be slightly higher than those obtained by LC-MS/MS (slope is 1.16), both methods can be considered statistically identical. Bland–Altman percent difference plot showed no concentration-dependant percent bias between both methods (see Fig. 2b).

Stability of serotonin in urine

Freshly collected urine samples were aliquoted and stored at various temperatures (20, 4, and at −20 °C). The concentration of serotonin was measured in triplicate on the day of collection and at various times thereafter, ranging from 2 days to 1 month. Serotonin was stable in all urine samples for at least 30 days if stored at 20, 4, or −20 °C (see Fig. S1 in the ESM). The drift acceptance criterion was ±15%.

Table 1 Analytical specificity and interferences

Substance	Concentration	Substance	Concentration
Major urinary components			
Ammonium chloride	10 mmol/L	Magnesium sulfate	15 mmol/L
Barbiturate	110 μmol/L	Oxalic acid	14 mmol/L
Bilirubin	260 μmol/L	Protein	600 mg/L
Calcium chloride	50 mmol/L	Sodium chloride	750 mmol/L
Creatinine	5 mmol/L	Sodium phosphate	100 mmol/L
Glucose	200 mmol/L	Urea	600 mmol/L
Hippuric acid	4 mmol/L	Urobilinogen	20 mmol/L
Structurally similar and other urinary components			
n-Acetylserotonin	575 nmol/L	3-Methoxytyramine	800 nmol/L
Alanine	600 μmol/L	Norepinephrine	120 nmol/L
3,4-Dihydroxyphenylglycol	180 nmol/L	Normetanephrine	7 μmol/L
3,4-Dihydroxyphenylacetic acid	3 μmol/L	β-Phenylethylamine	32 nmol/L
Dopamine	720 nmol/L	6-Sulfatoxymelatonin	100 nmol/L
Epinephrine	90 nmol/L	Tryptamine	375 nmol/L
Homovanillic acid	120 μmol/L	Tyramine	2.5 μmol/L
5-Hydroxyindoleacetic acid	44 μmol/L	Vanillylmandelic acid	60 μmol/L
Metanephrine	3 μmol/L		
Drugs/supplements[a]			
Acetominophen	480 mg/L	Isoprenaline	90 μg/L
Acetylsalicylic acid	6 g/L	Phenylalanine	11 mg/L
Ascorbic acid	800 mg/L	Phenylephrine	18 mg/L
Caffeine	2.8 mg/L	Salicylic acid	6 g/L
5-Hydroxytryptophan	15 mg/L	Theophylline	6 mg/L
Histidine	1.6 g/L	Tryptophan	90 mg/L
Isoetharine	3.6 mg/L	L-Tyrosine	4.8 g/L

[a] Maximum urinary levels in subjects under maximum-dose therapy

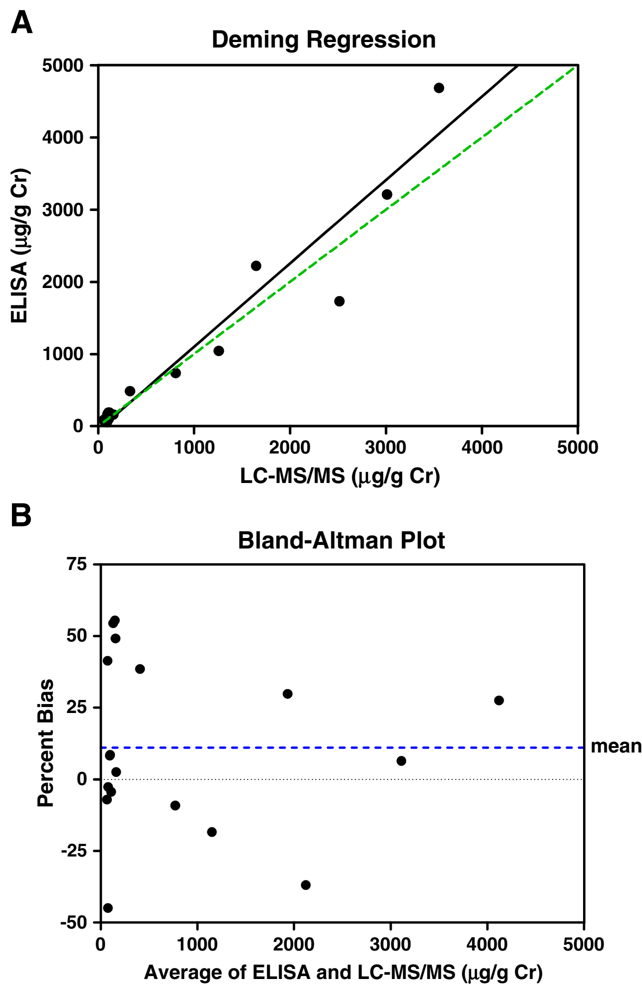


Fig. 2 Comparison of urinary serotonin measurements by ELISA and LC-MS/MS (n=18). (**A**) Deming regression analysis: slope is 1.156 (95% CIs, 0.9881 to 1.323); intercept is −53.8 (95% CIs, −280.9 to 173.4); Pearson correlation (r) is 0.965; (**B**) Bland–Altman plot of the percent bias against the average of the two methods. Mean percent bias is 11.0±29.9%

Reference interval

Urinary serotonin levels were assessed in 64 apparently healthy individuals. Their age range was 18–63 years with 43 females and 21 males. The parametric reference interval (95th percentile) [28, 29] was determined to be from 54 to 366 µg serotonin/g Cr.

Comparison of serotonin levels in 24-h urine and spot urine

We conducted a preliminary pre-clinical study to investigate the correlation between urinary serotonin levels in 24-h urine and spot urine collection (n=24). None of the participants (age 22–52 years) were using medications that could affect serotonin levels. The results of this study show a good correlation between spot urine collection and 24-h urine collection (see Fig. 3; p<0.01; r=0.91; n=24).

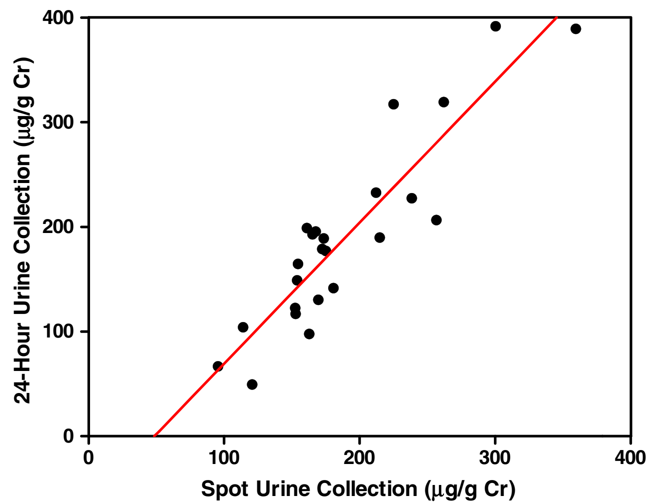


Fig. 3 Comparison of serotonin levels in 24-h urine and spot urine. The results suggest a good positive correlation between spot urine and 24-h urine collection as shown by linear regression (p<0.01; r=0.91; n=24). Linear regression analysis: slope is 1.34 (95% CIs, 1.07 to 1.63); intercept is −65.4 (95% CIs, −120.64 to −10.18)

Urinary serotonin as a potential biomarker of depression

In this retrospective study, serotonin was measured in urine samples collected from two population groups: depressed (n=60; 18 males and 42 females; age range, 18–65 years) and nondepressed subjects (n=60; 20 males and 40 females; age range, 18–61 years). The depressed group was identified based on self-reported depression symptoms and ICD-9 codes as established by a medical doctor (296–311). The nondepressed subjects were healthy according to the results of the HSCL-90. The individuals included in this study were not taking any medication or supplements for at least one month prior to urine collection. Based on two-

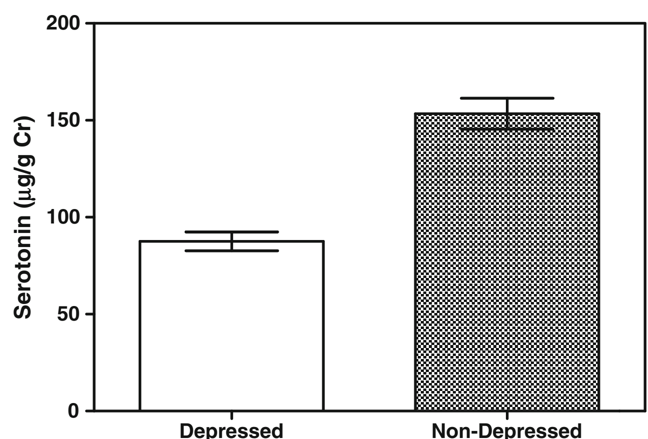
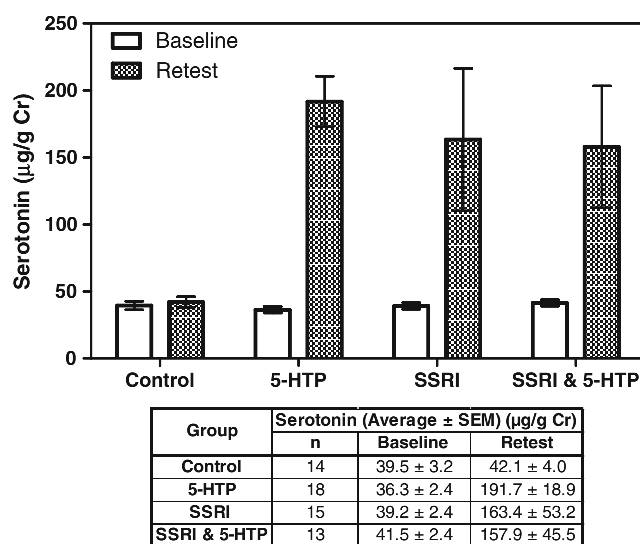


Fig. 4 Urinary serotonin levels (average±SEM) in depressed (n=60; 18 males and 42 females; age range, 18–65 years) and nondepressed subjects (n=60; 20 males and 40 females; age range, 18–61 years). Depressed group has significantly lower serotonin (87.53±4.89 µg/g Cr) than nondepressed group (153.38±7.99 µg/g Cr); two sample t test: p<0.001; t=−7.03; df=97.74; 95% CI=−84.72 to −47.27

sample *t* test ($p<0.001$; see Fig. 4), we found that the serotonin levels in urine from depressed subjects were significantly lower (87.53±4.89 μg/g Cr) than those in nondepressed subjects (153.38±7.99 μg/g Cr).

Urinary serotonin in patients under antidepressant therapy

The urinary serotonin levels were determined for four groups of depressed patients: (1) depressed patients using 5-HTP ($n=18$; ten males and eight females; age 20–81 years); (2) depressed patients using a SSRI ($n=15$; 4 males and 11 females; age range, 29–73 years); (3) depressed patients using a combination of a SSRI and 5-HTP (n=13; four male and nine females; age range, 20–73 years); and (4) depressed patients not using any treatment ($n=14$; 3 males and 11 females; age range, 20–73 years). For each group, urinary serotonin was tested as baseline and a retest. The retests were performed anywhere from 1 to 11 months after the baseline test, when subjects were still under treatment. We should note that the baseline tests were performed on subjects that were naïve to serotonin altering medications/supplements. The two-way ANOVA analysis demonstrated that the retested levels of serotonin in patients under antidepressant treatment were significantly higher than their corresponding baseline values (*p* value of <0.01, see Fig. 5). In addition, there were no significant differences in the retest serotonin levels between the three treatment groups. Finally, the urinary serotonin levels observed in depressed naïve subjects (Fig. 5) were lower in comparison



Group	Serotonin (Average ± SEM) (μg/g Cr)		
	n	Baseline	Retest
Control	14	39.5 ± 3.2	42.1 ± 4.0
5-HTP	18	36.3 ± 2.4	191.7 ± 18.9
SSRI	15	39.2 ± 2.4	163.4 ± 53.2
SSRI & 5-HTP	13	41.5 ± 2.4	157.9 ± 45.5

Fig. 5 Monitoring urinary serotonin in depressed patients under antidepressant therapy. Urinary serotonin levels at baseline and retest were determined in four study groups: (1) 5-HTP ($n=18$); (2) SSRI ($n=15$); (3) a combination of SSRI and 5-HTP ($n=13$); and (4) a control group without treatment ($n=14$). Subjects under antidepressant therapy had significantly higher serotonin compared with the untreated group ($p<0.01$; a two-way ANOVA)

to those found for depressed subjects with a history of medication use (Fig. 5).

Discussion

In this study, we reported the validation of a competitive ELISA for quantitative determination of urinary serotonin. The assay is characterized by low limits of quantification, excellent precision and analytical recovery. There was a good correlation ($R=0.965$) between urinary serotonin concentrations determined by the ELISA and by LC-MS/MS over the entire concentration range. The data indicated that the bias between the methods was not significant (mean percent bias is 11%). The differences between both methods could be attributed to variations in sample processing and calibration.

The analytical measurement range of the assay allows the direct measurement of serotonin in urine samples of the general population and in patients under antidepressant treatment where higher serotonin levels were observed. As described earlier (see "Materials and methods"), the serotonin ELISA is based on the use of a highly specific antibody (the highest crossreactivity observed for *N*-Ac-5HT (8.4%) is negligible). The analytical procedure including sample neutralization and glutaric aldehyde derivatizaion showed no significant interference by 44 structurally and non-structurally related urinary compounds. This allows for direct urinary analysis without any time-consuming and complicated sample purification as required by chromatographic methods. This greatly facilitates the routine monitoring of urinary serotonin in clinical practice.

The biological variability of urine specimen collection is a well recognized aspect in clinical diagnostics. For small studies, 24-h urine sample collection is preferred as it might provide a better average of serotonin secretion over a longer period of time. Compliance with this approach is however poor and often not clinically practical [32]. The results of the present comparative study between serotonin levels in 24-h urine collection and spot urine showed a good correlation ($p<0.01$; $r=0.91$; $n=24$). In addition, dividing the serotonin concentration by urinary Cr removes the factor of volume from the results of either spot or 24-h collection and is the most common method of correcting differences in hydration and/or intake of excessive fluids [33].

To evaluate whether the present ELISA method is useful for detecting variations in serotonin levels under pathological conditions, we compared the serotonin concentrations in urine samples of depressed individuals ($n=60$) to age and gender-matched apparently healthy controls ($n=60$). The results indicated that depressed subjects had significantly lower serotonin levels compared to nondepressed subjects ($p<0.001$). The etiology of the low (mainly peripheral) serotonin level in depressed subjects is unclear and whether

this reflects metabolic imbalance in serotonergic brain regions needs to be further elucidated. We demonstrated here for first time that low urinary serotonin levels can be related to depression. Reduced serotonin levels in platelets of depressed patients have been described before, but again, it was not clear whether the low serotonin was directly correlated to the development of depression and it reflects serotonin levels in the brain [34].

We have assessed the clinical utility of the serotonin immunoassay to monitor depressed patients under serotonin support treatment. The effect of the intake of 5-HTP, the rate-limiting precursor of serotonin, SSRI and a combination of both on urinary serotonin levels was investigated in three groups of depressed subjects. Urinary serotonin was elevated in all groups on antidepressant treatment but not in the untreated group. It has been reported that depressed subjects under SSRI treatment had significantly higher serotonin levels than nontreated depressed subjects [15], but until now the effect of SSRIs on serotonin clearance has been speculation [35]. In addition, it is accepted that the majority of 5-HTP administration is metabolized to serotonin in the kidney [36]. This might explain in part the significant increase of serotonin upon treatment. In this paper, we left the questions unanswered if treatment with 5-HTP, which can infiltrate the blood–brain barrier, actually restores an imbalance of serotonin in the brain.

In summary, the immunoassay procedure described in this work is relatively simple and requires only low-cost instrumentation (microtiter plate washer and reader) generally available in a clinical laboratory. In addition, the serotonin ELISA has the potential for higher throughput than LC assays. Therefore, the present immunoassay for serotonin can be considered an acceptable alternative for chromatographic methods. Furthermore, using this novel ELISA, we were able to establish that low urinary serotonin is indicative in people with depression. Finally, the ELISA can be successfully applied to monitor serotonin levels in patients under antidepressant treatment.

Acknowledgments We acknowledge the skillful technical assistance of Kathy Mork, Scott Howard and Saluja Mahamunuge. We would like to thank to Corena McManus from the R&D department of NeuroScience Inc. for the assistance in the clinical studies.

References

1. Marc DT, Ailts JW, Ailts-Campeau DC, Bull MJ, Kl O (2011) Neurotransmitters excreted in the urine as biomarkers of nervous system activity: validity and clinical applicability. Neurosci Biobehav Rev 35:635–644
2. Berry MD (2004) Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. J Neurochem 90:257–271
3. Kema IP, de Vries EG, Muskiet FA (2000) Clinical chemistry of serotonin and metabolites. J Chrom B 747:33–48
4. Nichols DE, Nichols CD (2008) Serotonin receptors. Chem Rev 108:1614–1641
5. Krishnan V, Nestler EJ (2010) Linking molecules to mood: new insight into the biology of depression. Am J Psychiatry 167:1305–1320
6. Cook IA (2008) Biomarkers in psychiatry: potentials pitfalls and pragmatics. Prim Psychiatr 15:54–59
7. Berman ME, Tracy JI, Coccaro EF (1997) The serotonin hypothesis of aggression revisited. Clin Psychol Rev 17:651–665
8. Risch SC, Nemeroff CB (1992) Neurochemical alterations of serotonergic neuronal systems in depression. J Clin Psychiatry 53:3–7
9. van Praag HM (1981) Management of depression with serotonin precursors. Biol Psychiatry 16:291–310
10. Cook EH, Leventhal BL (1996) The serotonin system in autism. Curr Opin Pediatr 8:348–354
11. Anderson GM (2002) Genetics of childhood disorders: XLV. Autism, part 4: serotonin in autism. J Am Acad Child Adolesc Psychiatr 41:1513–1516
12. Mulder EJ, Anderson GM, Kemperman RFJ, Oosterloo-Duinkerken A, Minderaa RB, Kema IP (2010) Urinary excretion of 5-hydroxyindoleacetic acid, serotonin and 6-sulphatoxymelatonin in normoserotonemic and hyperserotonemic austistic individuals. Neuropsychobiology 61:27–32
13. Turner EH, Loftis JM, Blackwell AD (2006) Serotonin a la carte: supplementation with the serotonin precursor 5-hydroxytryptophan. Pharmacol Therapeut 109:325–338
14. Russo S, Kema IP, Bosker F, Haavik J, Korf J (2009) Tryptophan as an evolutionarily conserved signal to brain serotonin: molecular evidence and psychiatric implications. World J Biol Psychiatry 10:258–268
15. Kotzailias N, Marker M, Jilma B (2004) Early effects of paroxetine on serotonin storage, plasma levels and urinary excretion. J Clin Psychopharmacol 24:536–539
16. Hyland K (2008) Clinical utility of monoamine neurotransmitter metabolite analysis in cerebrospinal fluid. Clin Chem 54:633–641
17. Anderson GM, Mefford IN, Tolliver TJ, Riddle MA, Ocame DM, Leckman JF, Cohen DJ (1990) Serotonin in human lumbar cerebrospinal fluid: a reassessment. Life Sci 46:247–255
18. De Jong WHA, Wilkens MHLI, De Vries EGE, Kema IP (2010) Automated mass spectrometric analysis of urinary and plasma serotonin. Anal Bioanal Chem 396:2609–2616
19. Pussard E, Guigueno N, Adam O, Giudicelli JF (1996) Validation of HPLC-amperometric detection to measure serotonin in plasma, platelets, whole blood, and urine. Clin Chem 42:1086–1091
20. Feldman JM (1986) Urinary serotonin in the diagnosis of carcinoid tumors. Clin Chem 32:840–844
21. Manz B, Kosfeld H, Harbauer G, Grill HJ, Pollow K (1985) Radioimmunoassay of human serum serotonin. J Clin Chem Clin Biochem 10:657–662
22. Chauveau J, Fert V, Morel AM, Delaage MA (1991) Rapid and specific enzyme immunoassay of serotonin. Clin Chem 37:1178–1184
23. Huisman H, Wynveen P, Setter PW (2010) Studies on the immune response and preparation of antibodies against a large panel of conjugated neurotransmitters and biogenic amines: specific polyclonal antibody response and tolerance. J Neurochem 112:829–841
24. Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH, Covi L (1974) The Hopkins Symptom Checklist (HSCL): a self-report symptom inventory. Behav Sci 19:1–15
25. Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH, Covi L (1974) The Hopkins Symptom Checklist (HSCL). A measure of primary symptom dimensions. Mod Probl Pharmacopsychiatr 7:79–110

26. Glass RM, Allan T, Uhlenhut EH, Kimball CP, Borinsetin DI (1978) Psychiatric screening in a medical clinic: an evaluation of a self-report inventory. Arch Gen Psychiatry 35:1189–1195
27. Huisman H, Wynveen P, Nichkova M, Kellermann G (2010) Novel ELISAs for screening of the biogenic amines GABA, glycine, beta-phenylethylamine, agmatine, and taurine using one derivatization procedure of whole urine samples. Anal Chem 82:6526–6533
28. Poulsen OM, Holst E, Christensen JM (1997) Calculation and application of coverage intervals for biological reference values. Pure Appl Chem 69(1601):1601–1611
29. Grasbeck R, Montalbetti N, PetitClerc C, Siest G, Solberg HE, Stamm D, Wilding P, Hjelm M, Leyssen MHJ, Tentori L, Verwilghen RL (1987) Approved recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. Clin Chim Acta 170:S13
30. Clinical and Laboratory Standards Institute. Evaluation of the linearity of quantitative measurement procedures: a statistical approach; approved guideline. CLSI document EP6, 2003.
31. Clinical and Laboratory Standards Institute. Interference testing in clinical chemistry; approved guideline (2005) Second Edition. CLSI document EP7-A2, vol. 25, no. 27.
32. Masi CM, Rickett EM, Hawkley LC, Cacioppo JT (2004) Gender and ethnic differences in urinary stress hormones: the population-based Chicago health, aging, and social relations study. J Appl Physiol 97:941–947
33. Ropero-Miller JD, Paget-Wilkes H, Doering PL, Goldberger BA (2000) Effect of oral creatinine supplementation on random urine creatinine, pH, and specific gravity measurements. Clin Chem 46:295–297
34. Le Quan-Bui KH, Plaisant O, Leboyer M, Gay C, Kamal L, Devynck MA, Meyer P (1984) Reduced platelet serotonin in depression. Psychiatr Res 13:129–139
35. Vaswani M, Linda FK, Ramesh S (2003) Role of selective serotonin reuptake inhibitors in psychiatry disorders: a comprehensive review. Prog Neuropsychopharmacol Biol Psychiatry 27:85–102
36. Li Kam Wa TC, Burns NJT, Williams BC, Freestone S, Lee MR (1995) Blood and urine 5-hydroxytrypthophan and 5-hydroxytryptamine levels after administration of two 5-hydroxytryptamine precursors in normal man. Br J Clin Pharmacol 39:327–329