A review of, “Neurotransmitters excreted in the urine as biomarkers of nervous system activity: Validity and clinical applicability”

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Approved for publication February 23, 2011
Journal: Neuroscience and behavioral reviews

Abstract: This writing reviews the paper, "Neurotransmitters excreted in the urine as biomarkers of nervous system activity: Validity and clinical applicability" herein referred to as "Marc et al 2010."

Purpose: The goal is to gauge the veracity of the scientific review and conclusions drawn by Marc et al. 2010.

Patients and methods: Literature cited by Marc et al. 2010 and related scientific literature was reviewed.

Results: In general, the cited references did not provide support for the claims made by Marc et al. 2010." It was found that the biomarker technology promoted by the paper is clinically unproven having no published clinical trials supporting applications in treatment of individual patients. The
very references cited by Marc et al. 2010 outline concerns with patient harm that may arise when clinically unproven biomarker technology is prematurely implemented. In review of other literature related to the topic it is clear that the use of spot baseline urinary neurotransmitter testing is not a valid biomarker sample collection technique due to randomness and lack of reproducibility in the individual.

**Conclusion:** The reader of the Marc et al. 2010 paper may be misled into believing that the cited references, the science presented, and the applications discussed are accurate and therefore valid. Biomarker applications advocated by the paper are based on an extension of a concept that in itself is invalid. The paper assumes that spot baseline urinary neurotransmitter testing is a valid testing approach for individual medical applications and therefore is a valid platform for biomarker applications. In fact, spot baseline urinary neurotransmitter testing of the monoamines serotonin, dopamine, norepinephrine, and epinephrine is not reproducible or valid and is clinically unproven in any application other than as a screening tool for a monoamine-secreting tumor.

**Keywords:** Urinary neurotransmitter testing, serotonin, dopamine, norepinephrine, epinephrine

**Introduction**

“Biomarker” is defined as, “A distinctive biological or biologically derived indicator (as a metabolite) of a process, event, or condition” (Webster’s Dictionary, 2010).

This manuscript is a review of a paper titled, “Neurotransmitters excreted in the urine as biomarkers of nervous system activity: Validity and clinical applicability” by David T. Marc, Joseph W. Ailts, Danielle C. Ailts Campeau, Michael J. Bull, Kelly L. Olson 2010. We hypothesize that Marc et al. 2010 have misinterpreted the published scientific data that forms the basis for clinical applications promoted by their paper.

Traditionally, concerns raised in reviewing a published manuscript may generate a letter to the editor. The scope and breadth of concerns identified in review of this paper gives rise to the need for a full manuscript to facilitate adequate scientific rebuttal and properly address the concerns raised.
The foundation of the Marc et al. 2010 paper is the “urinary neurotransmitter testing model.” This model advocates collection and use of spot baseline urine samples for monoamine neurotransmitter assays in clinical applications. Discussion of this model has been presented in previously published peer-reviewed literature covering the topic. Comprehensive review of the literature reveals that all published peer-reviewed original research articles to date have discredited the urinary neurotransmitter testing model. No original research articles could be found that support the approach. Results of spot baseline urinary neurotransmitter testing vary greatly from day to day and are not reproducible, being essentially random. Therefore, the use of this testing as advocated by Marc et al. 2010 does not form a valid basis for the applications promoted (Hinz et al. 2010b, 2011b).

There is no “original research” to support the validity of spot baseline urinary neurotransmitter testing, yet Marc et al. 2010 assumes, to the contrary, that the urinary neurotransmitter testing model is valid, then attempts to apply erroneous conclusions to the clinical care of individual medical patients without supporting clinical trials in place.

Previous literature scrutinized the urinary neurotransmitter testing model that is promoted by Marc et al. 2010. It is based on assertions that the neurotransmitters serotonin, dopamine, norepinephrine, and epinephrine (herein referred to as “the monoamines”) under normal conditions freely cross the blood-brain barrier then are filtered at the glomerulous and are placed directly into the final urine without further significant renal interaction. The “urinary neurotransmitter testing” model as discussed by Marc et al. 2010 uses these assertions regarding blood brain barrier and renal physiology to conclude that spot baseline urinary neurotransmitter testing is a direct assay of peripheral and central nervous system monoamine levels. Specifically the following is a quote from the Marc et al. 2010 paper, “In support of urinary neurotransmitter assessment, studies have demonstrated that intact neurotransmitter are transported from the CNS to the periphery, via specific BBB transporters, followed by renal filtration of neurotransmitters with subsequent excretion in the urine.” Marc et al. 2010 further notes, “Because urinary assessments are non-invasive, with the added advantage of enhanced stability compared to CSF or blood, the concept of
neurotransmitter measurements as an objective means to assess nervous system function serves as a viable option for the clinician addressing neuropsychiatric health concerns.

This position of the Marc et al. 2010 paper is in contrast to commonly accepted neuroscience and renal physiology. Volumes of peer-reviewed articles going back to the 1950s have consistently noted that under normal conditions the monoamines do not cross the blood-brain barrier (see item 2 of the Results section below). Renal physiology based literature clearly notes that under normal conditions peripheral monoamines filtered at the glomerulous are metabolized by the kidneys with no significant amount making it to the final urine. The Marc et al. 2010 paper stands in sharp contrast to the commonly accepted scientific research in renal physiology which notes that the monoamines found in the urine, under normal conditions, represent monoamines that are newly synthesized by structures of the kidneys; significant amounts have never been in the peripheral or central nervous systems. A 2011 article on this topic specifically notes, “While there have been attempts to integrate spot baseline urinary monoamine assays into treatment of peripheral or central neurotransmitter-associated disease states, diagnosis of neurotransmitter imbalances, and biomarker applications, significant differences in day-to-day reproducibility make this impossible given the known science as it exists today.” (Trachte et al. 2009, Stein et al. 2010, Hinz et al. 2009, 2010a, 2010b, 2010c, 2011a, 2011b, 2011c).

With the published proven premise that spot baseline urinary monoamine neurotransmitter testing is invalid due to significant differences from day to day in the same individual, arguments based on this spot testing model involving the blood-brain barrier and those regarding the source of urinary monoamines under normal conditions become moot. (Hinz et al. 2010b, 2011b) Conclusions drawn from invalid testing cannot be used for extended clinical applications as claimed in the Marc et al. 2010 paper.

Material and methods

Samples used for assay under the urinary neurotransmitter testing model are “spot baseline urinary monoamine neurotransmitter samples,” but urinary samples can be generated in several ways.
There are several different types of urine sample collection techniques. “Spot urine” is a single urine sample obtained at a specific time (Hinz et al., 2010b). The 12-hour or 24-hour urines are a collection of all urine excreted in the respective time period. The 24-hour urine is used when the total daily excretion of a substance by the kidneys into the final urine is studied, as is the case in the diagnosis of monoamine-secreting tumors. Collection of a 24-hour urine is burdensome and requires the subject to carry sample collection materials during all daily activities (Smythe et al., 1992). The 12-hour urine is rarely used, but it is discussed in one of the references found in the bibliography of the Marc et al 2010 paper. The primary reason given in that reference for collecting 12-hour samples was limitations due to urinary catheter changes that prevented 24-hour urine collection in a hospital setting (Delahanty et al., 2005).

The next differentiation of urinary testing is found in peer-reviewed writings. Urinary monoamines exist in two states: “the endogenous state” found when no amino acid precursors of the monoamines are being taken and “the competitive inhibition state” found when significant amounts of both serotonin and dopamine amino acid precursors are being taken. Obtaining urine samples in the endogenous state is known as “baseline testing” (Hinz et al. 2010a). The type of testing advocated and promoted in the Marc et al. 2010 paper for use in applications is “spot baseline urinary neurotransmitter testing.” (Stein et al. 2010, Hinz et al. 2009, 2010b, 2011b)

**Results**

In order to properly scrutinize the Marc et al. 2010 paper, peer-reviewed scientific literature was searched. The following five items of concern were identified and serve as the foundation in the discussion section of this paper.

**Item 1. Reproducibility of Test Results:**

Previously published peer-reviewed literature has demonstrated by matched pairs T-test that “spot baseline urinary neurotransmitter testing” of the monoamines serotonin, dopamine, norepinephrine, and epinephrine differs significantly from day to day when the individual patient is tested. These significantly different day-to-day results mean that spot baseline urinary neurotransmitter testing is not reproducible, not valid, and essentially a random process. With this
in mind, the spot urinary neurotransmitter testing model in individuals not suffering from a monoamine-secreting tumor has no biomarker applications. (Hinz et al. 2010b, 2011b)

Spot baseline urinary monoamine levels in a group of patients suffering from a specific disease may be increased relative to a control group, but the use of spot baseline laboratory testing for diagnosis and treatment of the individual in the clinical setting is unproven with no peer-reviewed original research to back it up. (Hinz et al. 2010b, 2011b)

It has been known for many years that spot baseline monoamine assay results in a group have markedly different clinical applications than do assays performed on an individual in the clinical setting. Examples of group observations include, but are not limited to:

- In a group of patients with carcinoid syndrome, spot group urinary serotonin levels are elevated. (Plonk et al. 1995, Siu et al. 1997)
- In a group of patients with pheochromocytoma, spot group urinary catecholamine (dopamine and/or norepinephrine) levels are elevated. (Symthe et al. 1992)

Monoamine-secreting tumors such as carcinoid syndrome and pheochromocytoma are associated with the highest reported individual and/or group urinary monoamine laboratory values in comparison with all the alleged clinical applications discussed in the Marc et al. 2010 paper. Even in these extreme monoamine disease states, spot baseline urinary neurotransmitter testing is nothing more than a screening tool to determine if definitive testing with a 24-hour urine sample may be indicated to formally make the diagnosis. (Plonk et al. 1995, Siu et al. 1997, Symthe et al. 1992) Spot urinary neurotransmitter testing under these conditions does not allow for diagnosis, predictability of treatment outcomes, or any of the other alleged benefits promoted by the Marc et al. 2010 paper (see section 4 below). The randomness of spot baseline test results differ significantly from one day to the next causing the lack of diagnostic ability and clinical specificity in treating these disease states (Hinz et al. 2010b, 2011b).

As noted in a previously published manuscript on the topic, “Due to the statistical difference in (spot) baseline monoamine assays in the same subject from day to day, an unlimited number of different neurotransmitter imbalances might theoretically be diagnosed with serial assays performed on many different days from the same subject. The assertion that baseline monoamine
assays can diagnose central nervous system, peripheral nervous system, and urinary neurotransmitter dysfunction is not supported on review of the scientific literature.” (Hinz et al. 2010b)

Trends seen in spot baseline urinary neurotransmitter testing in groups of patients suffering from a given disease state do not translate into clinical applications for individual patients for the reasons cited above and in peer-reviewed literature. With no clinical trials to the contrary, there is no way that these spot test results can have any meaning for individual patient care. Attempting to do so, as Marc et al. 2010 advocates, leads to multiple improper conclusions (Hinz et al. 2010b, 2011b).

**Item 2. The Blood-Brain Barrier:**

Marc et al. 2010 claims that the monoamines cross the blood-brain barrier. Previously published literature notes, “Significant challenges to the urinary neurotransmitter testing model include the widely recognized finding that serotonin and dopamine do not cross the blood–brain barrier under normal conditions. In support of applications for urinary serotonin and urinary dopamine assays, the urinary neurotransmitter testing model claims that serotonin and dopamine do cross the blood–brain barrier. This assertion is widely known to be untrue” (Hinz et al. 2010b).

The references cited in the bibliography of Marc et al. 2010 confirm these neurotransmitters do not cross the blood brain barrier contrary to the assertions of Marc et al. 2010. The following references cited in the bibliography of the Marc et al. 2010 paper demonstrate the opposite of what is asserted by paper.

- “There is no known transport mechanism of these monoamines that transports them across the blood-brain barrier” (Ohtsuki et al., 2004).
- Figure 2 of the Marc et al. 2010 paper illustrates that the monoamines do not cross the blood-brain barrier, but only enter into the endothelial cells where they are known to affect regulation. (Ohtsuki et al., 2004)
“Serotonin itself cannot cross the blood–brain barrier; therefore, the biosynthetic precursor of serotonin, 5-hydroxytryptophan (5-HTP), has been used as a dietary supplement to treat these serotonin-linked disorders” (Lynn-Bullock et. al. 2004).

There is an abundance of literature going back fifty years asserting that under normal conditions these monoamines do not cross the blood-brain barrier. The following is not intended to be exhaustive, but merely a sampling of literature over the years.


Sobocinska et al. 1987; Thomas et al. 1994; Elrod et al. 1997; Cameron et al. 2000; De Keyser et al. 2003; Rodrigues et al. 2009)


The references cited immediately above confirm that the peripheral and central monoamines are two distinct populations partitioned by the blood-brain barrier. These two pools of monoamines do not merge or mingle under normal conditions. The argument is made by Marc et al. 2010 that it is possible that under normal conditions the monoamines do merge or mingle by crossing the blood-brain barrier to the point of equilibrium between the two systems, but there is no literature, including the literature cited their references or elsewhere, that supports this assertion under normal conditions. Marc et al. 2010 claims that Figure 2 of the paper represents monoamines crossing the blood-brain barrier. In review of the reference cited by Marc et al. 2010 as being the foundation of its Figure 2, there is no claim in that reference that the monoamines cross the blood-brain barrier under normal conditions, only that they may enter into the endothelial cells of the blood-brain barrier then affect regulatory function. None of the Marc et al. 2010 paper references reviewed demonstrated or claimed that the monoamines freely cross the blood-brain barrier (Ohtsuki et al. 2004).

**Item 3. The Monoamines Filtered at the Glomerulous:**

Renal physiology clearly demonstrates that under normal conditions monoamines filtered at the glomerulous are metabolized by the kidneys. Significant amounts of filtered monoamines do not make it to the final urine. The monoamines found in the urine represent monoamines newly synthesized by structures found in the kidneys. Significant amounts of monoamines found in the
Contrary to the representations of Marc et al. 2010 under normal conditions the monoamines do not go directly into the urine after being filtered at the glomerulous. The urinary monoamines cannot be measured as peripheral and central biomarkers as they have never been in the peripheral or central circulation. (Stein et al. 2010, Hinz et al. 2009, 2010a, 2010b, 2010c, 2011a, 2011b, 2011c).

**Item 4. The Conclusions drawn by Marc et al. 2010:**

Based on the invalid assertions discussed in items 1 through 3 above in this section regarding the monoamines at the blood-brain barrier, the monoamines in the final urine, and disregarding the lack of reproducibility of the testing in an individual, the following are direct quotes and invalid conclusions.
<table>
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<tr>
<th>Marc et al. 2010 Claims</th>
<th>Comment</th>
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<tr>
<td>&quot;Of all the biological fluids that can be utilized, urinary neurotransmitter testing, due to its stability, sensitivity, and non-invasiveness, is the desired method to analyze nervous system function.&quot;</td>
<td>There is no evidence that urinary monoamine assays can determine nervous system function. (Hinz et al. 2010b, 2011b).</td>
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<td>&quot;Taken together, evidence suggests that neurotransmitters excreted in the urine may have a place in clinical practice as a biomarker of nervous system function to effectively assess disturbances and monitor treatment efficacy.&quot;</td>
<td>All previously published original research literature prior to the biomarker literature review has established that the urinary neurotransmitter testing model is not valid and reproducible; therefore, its use in biomarker applications is not valid. (Hinz et al. 2010b, 2011b).</td>
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<td>&quot;Due to the significant contribution of neurotransmitters to not only neurological functioning, but also endocrinological and immunological actions, clinicians and researchers are interested in the function and measurement of neurotransmitters as they have the potential to serve as clinically relevant biomarkers for specific disease states or to monitor treatment efficacy.&quot;</td>
<td>The spot baseline urinary neurotransmitter testing that the clinically unproven biomarker assertions are based on is contrary to the biomarker assertion found here. (Hinz et al. 2010b, 2011b).</td>
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<td>&quot;Specifically, studies suggested urinary neurotransmitter assessments might be a viable means to describe a disease state and to monitor therapeutic interventions.&quot;</td>
<td>There are no published clinical trials relating to treatment of individuals. There are some studies indicating that in groups of patients there may be a difference in urinary levels, but this has yet to be translated to the individual patient.</td>
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<td>&quot;Despite a historical absence of relevant biomarkers in the realm of clinical psychiatry, this format has expanded and neurotransmitters now serve as a primary target for the development of predictive or correlative biomarkers of nervous system function.&quot;</td>
<td>There is no basis for this comment. To assert that an invalid test is a “primary target” of research is inappropriate. Due to significant differences in testing from day to day spot baseline urinary neurotransmitter testing will never serve as a clinical biomarker in treatment of individual patients. (Hinz et al. 2010b, 2011b).</td>
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<td>&quot;The current body of literature provides evidence that neurotransmitters excreted in the urine may have a place in clinical practice as biomarkers of nervous system function.&quot;</td>
<td>This assertion on the part of the Marc et al. 2010 paper is contrary to all published literature relating to spot baseline monoamine assays which clearly demonstrated that spot baseline urinary monoamine assays are invalid and not reproducible. (Hinz et al. 2010b, 2011b).</td>
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<td>&quot;These findings illustrated the utilization of urinary neurotransmitter measurements to determine the underlying biochemical imbalances that exist in subjects with ADHD to ensure appropriate treatment selection and monitor treatment effectiveness.&quot;</td>
<td>In making this comment the Marc et al. 2010 paper is taking group laboratory observations and making a huge leap to clinical medical treatment of the individual without the benefit of any published clinical trials.</td>
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<td>&quot;These studies therefore, illustrate the significance of urinary neurotransmitter measurements in guidance of treatment selection and the prediction of efficacy.&quot;</td>
<td>With all evidence to the contrary, there is no evidence supporting this claim in the paper. All alleged references supporting this assertion by the Marc et al. 2010 paper appear to be</td>
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“While unconfirmed, these findings demonstrate how the renal contribution to overall neurotransmitter excretion can vary for each neurotransmitter and highlights the value of urine as a means to evaluate system-wide disturbances in neurotransmitter function.”

The Marc et al. 2010 paper correctly notes “While unconfirmed…” but it inappropriately notes, with no original research support, that invalid spot baseline urinary neurotransmitter testing is a “…means to evaluate system-wide disturbances in neurotransmitter function.” (Hinz et al. 2010b, 2011b).

“In line with these studies, research has described the utility of urinary neurotransmitter analysis in bipolar depression and subtypes of unipolar depression.”

Once again the paper is taking group laboratory observations and applying them to individual treatment results in clinic without proper clinical trials in place. While, in fact, there may be an elevation in group laboratory levels, the fact still remains that testing of the individual is invalid due to lack of reproducibility. (Hinz et al. 2010b, 2011b).

“As of now, the use of urinary testing of neurotransmitters as a biomarker may be best applied to guide therapeutic decisions and assist practitioners in clinical settings to effectively predict treatment responses.”

The Marc et al. 2010 paper is once again erroneously applying the invalid spot baseline urinary monoamine testing to guide the direction of medical treatment. (Hinz et al. 2010b, 2011b).

“Because urinary assessments are non-invasive, with the added advantage of enhanced stability compared to CSF or blood, the concept of neurotransmitter measurements as an objective means to assess nervous system function serves as a viable option for the clinician addressing neuropsychiatric health concerns.”

The Marc et al. 2010 paper has taken group observations in laboratory testing and used a test that is invalid due to lack of reproducibility without proper clinical trails in place or published, and makes statements such as this which totally lack the supporting scientific literature on any level. An objective review of literature should reflect what the literature reports. (Hinz et al. 2010b, 2011b).

Table 1: Assertions of Marc et al. 2010 with commentary response.

The lack of reproducibility of the testing with the individual and misrepresentation of published literature as further demonstrated below make all of the above conclusions invalid.

**Item 5. Reporting of References**

In the course of this review, numerous references identified in the footnotes and bibliography of the Marc et al. 2010 paper stated the exact opposite of what the paper is claiming they said, or the paper has made claims that cannot be found in the cited references. Charts 1 and 2 contain a few examples of the numerous divergences found in the paper:
<table>
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<th>Chart 1: Author statement versus reference cited</th>
<th>The literature cited</th>
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<td><strong>The statement by Marc et al.</strong></td>
<td>Lepschy et al., 2008 notes, “This represents the first step in the development of a reliable, non-invasive quantification of epinephrine and norepinephrine to monitor sympathoadrenomedullary activity, although promising results for the development of a non-invasive method were found only for the chicken...In conclusion, establishing a non-invasive method for measuring CA (catecholamine) in feces seems to be feasible only in the chicken.” Based upon a study of urine and feces in mice, rats, and chickens that found chicken feces testing of norepinephrine and epinephrine to be the only feasible option, the Marc et al. 2010 paper authors have incorrectly asserted that “Urine...has been the preferred bodily fluid for neurotransmitter measurements” in humans.</td>
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<tr>
<td>The paper on page 2 asserts, “Urine, due to its non-invasive method of collection, and being the primary method of neurotransmitter elimination, has been the preferred bodily fluid for neurotransmitter measurements (Lepschy et al., 2008).”</td>
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<td>The paper on page 2 asserts, “The BBB transporters that shuttle neurotransmitters and their metabolites out of the CNS are depicted in Fig. 2. Studies have documented the presence of a number of major neurotransmitter transporters at the BBB, such as the ... norepinephrine transporter (NET) (Wakayama et al., 2002).”</td>
<td>This statement is misleading. Norepinephrine is transported out of the brain to the endothelial cells of the blood-brain barrier (BBB), but does not cross the BBB. The article does not discuss these molecules crossing the BBB. The article notes, “The physiologic role of NET and SERT at the BBB appears to involve monoamine inactivation around the brain capillaries, and plays an important role in the regulation of BBB function by monoamines.”</td>
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<td>The paper on page 3 asserts, “Monoamine neurotransmitters are excreted by ultrafiltration from arterial blood into the glomeruli, secreted in the proximal tubules, subsequently distributed through the collecting duct to the urinary bladder and excreted in the urine” (Graefe et al. 1997).</td>
<td>The Graefe reference does not refer to the process or word “ultrafiltration” anywhere in the manuscript. The Graefe reference also notes, “It is well established that the main source of urinary dopamine is dopamine formed in proximal tubular cells of the kidney.”</td>
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<td>The paper on page 3 asserts, “Studies have identified the presence of neurotransmitter transport molecules on nephrons that move neurotransmitters from the extracellular space to abolish their biological actions and actively excrete them in the urine” (Hayer-Zillgen et al. 2002).</td>
<td>Nowhere in Hayer-Zillgen et al. 2002 is there any reference to urine or kidney functions. The writing is an in vitro study of cloned transporters.</td>
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<td>The paper on page 4 asserts, “Therefore, urinary neurotransmitter excretion mediated by OCT, is dependent on circulating neurotransmitter concentrations” (Chekhonin et al., 2000; Lynn-Bullock, 2004).</td>
<td>In the Chekhonin and Lynn-Bullock references the words “OCT,” “organic” and “cation” do not appear anywhere. There is no discussion of neurotransmitter excretion modulated by the OCT as asserted by the paper being reviewed.</td>
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**Chart 1:** A review of the accuracy and veracity of statements made by the authors.
<table>
<thead>
<tr>
<th>Marc et al. statement</th>
<th>The literature cited</th>
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<tr>
<td>The paper on page 6 asserts, “Specifically, studies suggested urinary neurotransmitter assessments might be a viable means to describe a disease state and to monitor therapeutic interventions” (Hughes et al., 2004).</td>
<td>This is a study of patients with depression, with 24-hour urines collected, not the spot urines as discussed by the biomarker article. While as a group the 24-hour norepinephrine levels may be elevated, individual testing is not valid because of day-to-day fluctuations in norepinephrine levels. Nowhere in the reference cited does it discuss 24-hour norepinephrine testing as having the ability to describe a disease state or monitor therapeutic interventions as asserted. The authors refer to the broad term “neurotransmitter,” yet the paper only discusses norepinephrine in a group application.</td>
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<td>The paper on page 6 asserts, “In line with these studies, research has described the utility of urinary neurotransmitter analysis in bipolar depression” (Koslow et al., 1983).</td>
<td>Contrary to the assertions of the authors, the Koslow reference avers, “Therefore, it cannot be stated that our findings are influenced either by the clinical phenomena observed in affective disorders or their origin; they may in fact only be epiphenomena of these disorders.” There is no discussion of the utility of urinary neurotransmitter analysis as asserted by the authors.</td>
</tr>
<tr>
<td>The paper on page 6 asserts, “As of now, the use of urinary testing of neurotransmitters as a biomarker may be best applied to guide therapeutic decisions and assist practitioners in clinical settings to effectively predict treatment responses” (Holsboer, 2008).</td>
<td>The Holsboer reference relates to genetic biomarkers and makes no reference to the urine or other renal processes.</td>
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<td>The paper, beginning on page 8 asserts, “Urinary neurotransmitter measurements were initially utilized to diagnose pheochromocytoma, but with progressive research, neurotransmitter testing has shown promise as a method to evaluate patients with psychiatric and inflammatory disorders” (Delahanty et al., 2005).</td>
<td>Review of the Delahanty reference reveals that it has nothing to do with evaluating patients with psychiatric and inflammatory disorders. It is a prospective study attempting to determine, in patients without post-traumatic stress disorder, which patients may be at risk for developing the disease. The study was performed under very narrow conditions of a trauma center environment, and did not use spot urine testing as referred to in the Marc et al. 2010 paper.</td>
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**Chart 2:** A review of the accuracy and veracity of statements made by Marc et al. 2010.

The Marc et al. 2010 paper lists 132 references in the bibliography. Not all of the references were reviewed for this paper. Finding the examples used in Charts 1 and 2 was not difficult. Approximately 95% of the reference citations reviewed contained inaccuracies of the caliber discussed in Charts 1 and 2. The process of selecting quotes from the Marc et al. 2010 paper for
charts 1 and 2 was as follows. The statements found in the left column were identified in the Marc et al. 2010 paper, and then thirteen papers from the references cited were obtained and reviewed to generate the twelve direct literature quotes and comments listed in the right column. The thirteenth paper, which was not used, contained similar inaccuracies, but the scientific discussion required was lengthy causing it to be removed from our discussion consideration.

**Discussion**

A primary flaw of the Marc et al. 2010 paper involves improperly applying the results reported for 24-hour urine samples to this spot baseline urinary neurotransmitter testing, and then claiming that the 24-hour result conclusions can be freely applied in individual clinical applications based on 24-hour urine studies when only spot baseline urine was collected.

All of the literature located and reviewed for this paper, including the references cited by Marc et al. 2010 bibliography, indicates that under normal conditions monoamines do not cross the blood-brain barrier. (See item 2 of the Results section) Review of renal physiology indicates that under normal conditions monoamines filtered at the glomerulous do not make it to the final urine in significant amounts secondary to being metabolized by the kidneys. Under normal conditions the baseline urinary monoamine levels primarily represent monoamines that are newly synthesized by the kidneys and have not been in the peripheral or central systems. (Stein et al. 2010, Hinz et al. 2009, 2010a, 2010b, 2010c, 2011a, 2011b, 2011c).

Arguments relating to the blood-brain barrier along with renal metabolism and source of urinary monoamines become moot when it is realized that the spot baseline urinary neurotransmitter testing advocated by Marc et al. 2010 differs significantly from day to day in the individual, is not reproducible and therefore is essentially random as verified by matched pairs T-test statistical scrutiny previously presented in novel original research writings. This same invalid spot baseline urinary neurotransmitter testing is the basis for the unproven biomarker applications promoted by the paper. There are no reported or known clinical studies supporting the individual medical treatment applications advocated (Hinz et al. 2010b, 2011b).

One of the references cited by the Marc et al. 2010 paper notes the following:
“Perhaps most worrisome is the problem of premature clinical application (of biomarkers), both because of the risk for harm to patients (misdirected in treatment decisions) and for the cynicism about biomarkers in general this engenders; still, the need for useful biomarkers is so great that sometimes enthusiasm and optimism may overtake consideration of results from carefully conducted controlled clinical trials. To paraphrase the film Jerry Maguire, “show me the data!” must be the watchword if clinicians are to make prudent choices for their patients.” (Cook, 2008)

We assert that Cook et al. 2008, in citing “premature clinical applications” of biomarker testing, is discussing the exact problem associated with the Marc et al. 2010 paper. Careful review of the references cited reveals no definitive clinical trials or support regarding use of spot baseline urinary neurotransmitter testing in biomarker applications for treatment of individuals in the clinical setting. The Cook reference is correct that premature clinical application of an unproven biomarker application can lead to risk of harm to patients. Examples of the harm supported under the Cook writing include, but are not limited to:

- A diagnosis of a false normal state when disease exists.
- Misdiagnosis of disease states.
- Medical treatment decisions that make the disease state worse.
- Can induce unnecessary treatment.
- Can delay implementing available valid and beneficial treatment.
- Can give false hope where none exists leading to distress when this is realized.
- Interference with the doctor-patient relationship when expected results promoted by the laboratory do not turn out as advertised due to formulated unrealistic expectations of care.

Having no clinical trials with which to test their hypothesis or to allow verification through reproducibility by others and no published literature to support their conclusions, these unproven biomarker applications are not appropriate for clinical medicine at this time. It would appear that
the Marc et al. 2010 paper has taken group laboratory observations and liberally drawn conclusions regarding patient care outcomes without proper foundation.

The science of monoamine renal physiology is exceptionally complex, yet at every turn Marc et al. 2010 have created a simplified version that has nothing to do with the known science.

**Conclusion**

Spot baseline urinary neurotransmitter testing of the type advocated by the Marc et al. 2010 paper has been discredited in the literature in the past for lack of reproducibility and validity. Spot baseline urinary neurotransmitter assays differ significantly from baseline assays performed on a different day in the same subject. There is currently no scientific basis, value, or predictability in obtaining this spot baseline urinary neurotransmitter testing of the monoamines. The spot baseline urinary neurotransmitter laboratory testing applications reviewed in the Marc et al. 2010 paper have no clinical use other than as a screening tool for monoamine-secreting tumors, and have a potential to cause a significant negative impact on health care if suggested clinically unproven applications of the Marc et al 2010 paper are implemented (Hinz et al. 2010b, 2011b).

Marc et al. 2010 did not report the primary literature accurately, making a number of misleading statements and drawing unsupported conclusions.

Doctors have a right to believe that the laboratory testing being performed and the interpretation suggested is being done with the utmost honesty, integrity and reproducibility on a day-to-day basis. They need to be able to trust the laboratory to provide accurate, reproducible data along with accurate interpretation assistance. Scientifically invalid promotions and extensions of applications and non-reproducible laboratory testing challenge the veracity and honorability expected by doctors and other health care providers in managing patient health care. The methodology and the promotion of applications found in this particular instance have every probability of creating a serious health risk, with potential negative outcomes for patients’ health.

This review is intended to bring scrutiny to the Marc et al. 2010 paper. It is the goal of this paper to invite analysis of this topic, spark interest, and promote insight into the proper science of urinary monoamine assays.
Acknowledgments/Disclosures

MH discloses ownership of DBS Labs, Duluth, MN. TU discloses directorship of DBS Labs, Duluth, MN. AS reports no disclosures.

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