REFERENCE ARTICLES

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References That Support Urinary Neurotransmitter Testing

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Urinary histamine metabolite elevations during experimental influenza infection. Skoner DP, Gentile DA, Fireman P, Cordoro K, Doyle WJ. (2001). *Ann Allergy Asthma Immunol*, 87(4):303-306. PMID 11686422

Publications by NeuroScience, Inc. and Pharmasan labs, Inc.

An attachment based approach to child custody evaluation: A case study. Purvis KB, McKenzie LB, Kellermann G, and Cross DR. (2010). *Journal of Child Custody*, 7(1): 45-60.

Abstract: This child custody consultation, conducted in a rural Texas community, addressed the immediate placement needs of a 5-year-old female, Jamie. The child had been in foster care for 1 year, and her biological mother was seeking reinstatement of full custody. During supervised visitation sessions, Jamie displayed behaviors that are associated with a disorganized attachment style and/or seizure activity, while the mother exhibited behaviors indicative of an insecure attachment style and the inability to be emotionally available to her child. The Adult Attachment Inventory was administered to the mother as a measure of attachment while Jamie was tested for neurotransmitter activity at baseline and immediately after visitation. The results of these objective measures are presented and the implications for their use in child custody cases are discussed.

Evaluation of a novel ELISA for serotonin: urinary serotonin as a potential biomarker for depression. Nichkova M, Huisman H, Wynveen P, Marc D, Olson K, Kellermann G. (2011). *Analytical and Bioanalytical Chemistry*, 399 (1): ONLINE FIRST DOI: 10.1007/s00216-011-5583-1. PMID: 22160204

Abstract Depression is a common disorder with physical and psychological manifestations often associated with low serotonin. Since noninvasive diagnostic tools for depresson 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 derived urine samples. Assay performance was evaluated and applied to clinical samples. The analytical range of the assay was from 6.7 to 425 µg serotoinin/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 serotoinin 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 of 5-hydroxy-tryptophane and/or selective serotonin re-uptake inhibitor (p<0.01). The present ELISA provides a convenient and robust metohod for monitoring urinary serotonin. It is suitable to monitor serotonin imbalances and may be particularly helpful in evaluating antidepressant therapies.

Neurotransmitters excreted in the urine as biomarkers of nervous system activity: validity and clinical applicability. Marc DT, Ailts JW, Ailts-Campeau DC, Bull MJ, & Olson KL. (2010). *Neuroscience Biobehavioral Rev*, 35.635-644. PMID: 20696183.

Abstract: Strategies for managing the nervous system are numerous while methods of evaluating the nervous system are limited. Given the physiological importance of neurotransmitters as signaling molecules in the nervous system, the measurement of neurotransmitters has significant potential as a clinical tool. 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. Increasing use of this technology in a clinical setting demands a review of its feasibility, utility, and clinical value. We review the current body of literature pertaining to the mechanism of neurotransmitter transport across the blood-brain barrier as well as neurotransmitter filtration and excretion by the kidneys. In addition, this review summarizes the historical use of urinary neurotransmitter assessment to diagnose pheochromocytoma. Early research also correlated urinary assessment of neurotransmitters to various clinical symptoms and treatments of which we present research only for depression, ADHD, and inflammation because of the abundant amount of research in these areas. Finally, we review the limitations and challenges of urinary neurotransmitter testing. 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.

Novel ELISAs for screening of the biogenic amines GABA, glycine, β-phenylethylamine, agmatine, and taurine using one derivatization procedure of whole urine samples. Huisman H, Wynveen P, Nichkova M, & Kellermann G. (2010). *Journal of Analytical Chemistry*, 82(15), 6526–6533. PMID: 20586417

Abstract: The inhibitory neurotransmitters GABA, glycine and agmatine and neuromodulators beta-phenylethylamine (beta-PEA) and taurine are important biogenic amines of the sympathetic and parasympathetic nervous systems in the body. Abnormalities in the metabolism of these biomarkers have been implicated in a vast number of neurological diseases. Novel competitive immunoassays, using one unique whole urine derivatization procedure applicable for all five biomarkers, have been developed. The determination of these biomarkers was highly reproducible: the coefficient of variance of inter- and intra-assay variation is between 3.9% and 9.8% for all assays. The assays show a good linearity in urine samples within the range of 100-400 mg Cr/dL and specificity when urine samples are spiked with biogenic amines. The recoveries are between 76 and 154%. The correlation between HPLC and ELISA for glycine and taurine (n = 10) showed regression coefficients of 0.97 and 0.98, respectively. An in vivo study on the urinary clearance of beta-PEA, agmatine and taurine after oral intake by healthy individuals demonstrated the specificity and clinical significance of these new immunoassays. The immunoassays are useful for clinical and basic research where a fast and accurate assay for the screening of biogenic amines in urine is required, without preclearance of the sample.

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. Huisman H, Wynveen P, & Setter PW. (2010). *Journal of Neurochemistry*, 112, 840–852. PMID: 19912471

Abstract: We described the production and characterization of antibodies against three important groups of neuro-active haptens, e.g., neurotransmitters and biogenic amines. First, from the tryptophane metabolic pathway: tryptamine, serotonin, 5-hydroxy-indole acetic acid, and melatonin. Secondly, the tyrosine metabolic pathway: tyramine, dopamine, dihydroxyphenyl acetic acid, and norepinephrine. Thirdly, antibodies against excitatory and inhibitory neurotransmitters: glycine, glutamate, glutamine, and GABA. Immunogenic conjugates were prepared after linking haptens to carrier proteins. Most antibodies displayed high specificity against corresponding neuro-active haptens conjugated in vitro and in situ in biological specimens, but not to closely related conjugated metabolites, precursors, pharmaceuticals, agonists, antagonists, or free neuro-active haptens. Conjugated norepinephrine was highly tolerant in different animal species and produced incidentally a short specific antibody response.

A randomized targeted amino acid therapy with behaviorally at-risk adopted children. Cross DR, Kellermann G, McKenzie LB, Purvis KB, Hill GJ, & Huisman H. (2010) *Child care health and development*, 37(5): 671-678. PMID: 21166834

Abstract: Background: Increasing numbers of children are at-risk for behavioral and emotional disorders, a phenomenon contributing to increased use of pharmacological interventions for pediatric clients. Adverse side effects and other risks associated with pharmacological approaches have helped fuel interest in nutritional interventions for behaviorally at-risk children. Methods: The current randomized clinical trial evaluates the efficacy of a neurochemical intervention involving the glutamine and glutamate analogue L-theanine and 5-hydroxytryptophan, the precursor for serotonin, with children adopted from traumatic backgrounds. Results: Results include significant increases in urinary levels of the biomarkers for serotonin and gamma-aminobutyric acid, coupled with significant decreases in parent reports of the children's behavior problems. Conclusions: While further research is needed, these initial findings are encouraging and are consistent with a growing number of studies indicating the efficacy of nutritional approaches to help behaviorally at-risk children.

Publications Using Pharmasan Labs, Inc. Assays from Outside Researchers

Enter the PubMed I.D.# (PMID) in the search bar at Pubmed.gov to view abstracts.

A compromise phase position for permanent night shift workers: circadian phase after two night shifts with scheduled sleep and light/dark exposure. Lee C, Smith MR, Eastman CI. (2006). *Chronobiol Int,* 23:859-75. PMID: 16887753

A late wake time phase delays the human dim light melatonin rhythm. Burgess HJ, Eastman CI. (2006). *Neurosci Lett,* 395:191-5. PMID: 16309837

A three pulse phase response curve to three milligrams of melatonin in humans. Burgess HJ, Revell VL, Eastman CI. (2008). *J Physiol,* 586:639-47. PMID: 18006583

Adaptation of human pineal melatonin suppression by recent photic history. Smith KA, Schoen MW, Czeisler CA. (2004). *J Clin Endocrinol Metab*, 89:3610-4. PMID: 15240654

Advancing circadian rhythms before eastward flight: a strategy to prevent or reduce jet lag. Eastman CI, Gazda CJ, Burgess HJ, Crowley SJ, Fogg LF. (2005). *Sleep*, 28:33-44. PMID: 15700719

Advancing human circadian rhythms with afternoon melatonin and morning intermittent bright light. Revell VL, Burgess HJ, Gazda CJ, Smith MR, Fogg LF, Eastman CI. (2006). *J Clin Endocrinol Metab*, 91:54-9. PMID: 16263827

Binaural beat technology in humans: a pilot study to assess psychologic and physiologic effects. Wahbeh H, Calabrese C, and Zwickey HJ. Altern. (2007). *Complement Med*, 13(1): 25-32. PMID: 17309374

Circadian phase determined from melatonin profiles is reproducible after 1 wk in subjects who sleep later on weekends. Revell VL, Kim H, Tseng CY, Crowley SJ, Eastman CI. (2005). *J Pineal Res*, 39:195-200. PMID: 16098098

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The dim light melatonin onset following fixed and free sleep schedules. Burgess HJ, Eastman CI. (2005). *J Sleep Res*,14:229-37. PMID: 16120097

Early versus late bedtimes phase shift the human dim light melatonin rhythm despite a fixed morning lights on time. Burgess HJ, Eastman CI. (2004). *Neurosci Lett*, 356:115-8. PMID: 14746877

"After all, the ultimate goal of all research is not objectivity, but truth."

-- Helen Deutsch

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NeuroScience, Inc. and Pharmasan Labs, Inc. Research Collaborations

In our goal of improving health through understanding of the NEI Supersystem©, NeuroScience, Inc. and Pharmasan labs, Inc. have collaborated with many reputable researchers.

Abbott Northwestern Hospital

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National College of Natural Medicine, Helfgott Research Institute

Rensselaer Polytechnic Institute

Rush University Medical Center

Texas Christian University

University of Chicago

University of Cincinnati / Wright State University

University of Maryland, Center for Integrative Medicine

University of Minnesota / Regions Hospital

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CLIA ID#52D0914898

New York State certification.

Pharmasan Labs, Inc., has received New York State certification:

New York State Department of Health
PFI #7426

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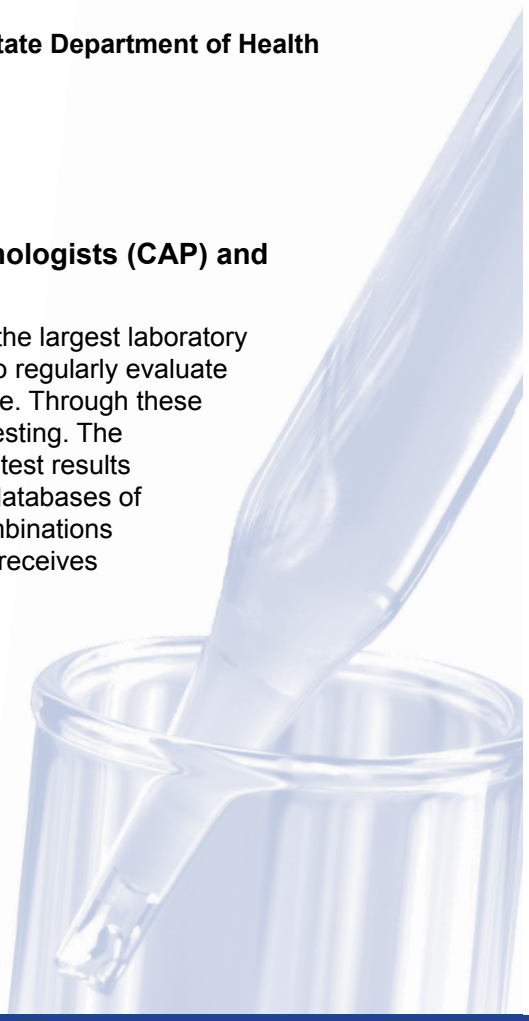
The CAP Surveys and Anatomic Pathology Education/EXCEL Programs are the largest laboratory peer comparison programs in the world. These programs allow laboratories to regularly evaluate their performance and improve the accuracy of the patient results they provide. Through these programs, individual laboratories are provided with unknown specimens for testing. The participants analyze the specimens and return the results for evaluation. Lab test results are evaluated using comparable peer groups from the most comprehensive databases of laboratories. Labs are compared to the most relevant instrument/reagent combinations to accurately assess their performance. In turn, each participating laboratory receives a report of their performance as well as a report summarizing the results of all participating laboratories. This information can be used to improve performance in order to provide the best patient care.



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