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| **About the Measure** |
| **Protocol Id** | 720401 |
| **Domain:** | Tobacco Regulatory Research - Host: Biobehavioral  |
| **Measure:** | NNAL in Urine  |
| **Definition:** | An assay to measure NNAL, the primary metabolite of NNK.  |
| **Purpose:** | To measure exposure to tobacco-specific nitrosamine carcinogens.  |
| **Essential PhenX Protocols:** | Current Age [10101]Ethnicity and Race [11901]Sex Assigned at Birth [11601]Gender Identity [11801]Cigarette Smoking Status - Adolescent [30603]Cigarette Smoking Status - Adult [30604]  |
| **Related PhenX Protocols:** | Cotinine in Serum [720201]  |
| **Measure Release Date:** | February 20, 2015  |

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| **About the Protocol** |
| **Protocol Release Date:** | February 20, 2015  |
| **Protocol Review Date:** | February 20, 2015  |
| **PhenX Protocol Name:** | NNAL in Urine  |
| **Protocol Name From Source:** | National Health and Nutrition Examination Survey (NHANES), Laboratory Procedure Manual, NNAL In Urine, 2011-2012  |
| **Protocol Availability:** | Available  |
| **Keywords:** | biomarker; NNAL; urine; tobacco-specific carcinogen; TSNA; tobacco smoke exposure; tobacco product exposure; tobacco chemical exposure; smokeless tobacco exposure; Centers for Disease Control and Prevention; National Health and Nutrition Examination Survey; NHANES; laboratory protocol; second hand smoke; secondhand smoke; second-hand smoke  |
| **Description:** | Detailed laboratory protocol for measuring urinary NNAL as used by the Centers for Disease Control and Prevention to analyze urine specimens from the National Health and Nutrition Examination Survey (NHANES). |
| **Specific Instructions:** | Because of the sensitive nature of this assay, analysts cannot be actively using tobacco products, and measurements must be performed in a smoke-free environment.Safety Precautions: Wear eye protection and suitable protective clothing when using this method to extract and process samples. |
| **Protocol:** | NNAL is measured by using liquid chromatography linked to tandem mass spectrometry (LC-MS/MS). For "total" NNAL assays, the urine sample is fortified with an NNAL-13C6 internal standard, and then hydrolyzed using β-glucuronidase in incubations for at least 24 hours. The samples are then extracted on a supported liquid extraction cartridge, followed by liquid-liquid extraction and finally cleaned up on a specially-designed solid-phase molecularly-imprinted polymer (MIP) column, after which the analyte is eluted and analyzed by LC/MS/MS, monitoring the m/z 210->180 native, and m/z 216->186 internal standard transition ions. NNAL concentrations are derived from the ratio of the integrated peaks of native to labeled ions by comparison to a standard calibration curve. Free NNAL measurements are conducted in a similar manner, but with the omission of prior enzymatic hydrolysis. Bound NNAL (e.g. NNAL-Gluc) may be estimated from the difference of (Total NNAL - Free NNAL). Prior to assaying each run of unknowns, the results from standard analyses are reviewed for acceptable accuracy, precision and instrument sensitivity. The results from the 20 calibration standards analyzed prior to each run are reviewed daily. Acceptable back-calculated values for standards above the detection limit are typically in the range of nominal concentration ± 10%. The limit of detection is 0.6 pg/mL. Liquid chromatography-tandem mass spectrometry (LC-MS-MS) is the preferred method to accurately measure NNAL in urine samples (see source references for details). |
| **Selection Rationale:** | 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is a major metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), the most potent pulmonary carcinogen of all tobacco-specific *n*-nitrosamines (TSNA). TSNAs are known to be carcinogenic to many animal species and are believed to be carcinogenic to humans as well. These carcinogens are of special significance because they combine an inherent potent pulmonary carcinogenic potential with a high degree of tobacco-exposure specificity because they can be found only in tobacco and tobacco smoke. Measuring NNAL in urine is a reliable way to determine exposure to NNK for smokers, for nonsmokers exposed to environmental tobacco smoke (ETS), and for people who use smokeless tobacco products (e.g., chewing tobacco, snuff). This protocol was used to measure urinary NNAL in NHANES from 2007 to 2014. |
| **Source:** | CDC Laboratory Procedure Manual for NNAL In Urine, [link[www.cdc.gov/nchs/data/nhanes/nhanes\_11\_12/2011-12\_Laboratory\_Procedures\_Manual.pdf|Laboratory Procedures Manual]] |
| **Language** | English  |
| **Participant:** | All participants who can provide urine sample. See section 4 of the "CDC Laboratory Procedure Manual for NNAL in Urine."  |
| **Personnel and Training Required:** | Laboratory training in the use of liquid chromatography and tandem mass spectrometry is required. All analysts must be CLIA-certified and demonstrate proficiency in the analysis before handling samples. |
| **Equipment Needs:** | This method requires high-performance liquid chromatography and tandem mass spectrometry as detailed in the National Health and Nutrition Examination Survey (NHANES) [link[www.cdc.gov/nchs/data/nhanes/nhanes\_11\_12/2011-12\_Laboratory\_Procedures\_Manual.pdf|Laboratory Procedures Manual]] |
| **Standards** |  |
| **General References:** | Hecht, S. S. (2002). Human urinary carcinogen metabolites: Biomarkers for investigating tobacco and cancer. *Carcinogenesis, 23*, 907-922.Hecht, S. S. (1999). Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute, 91*, 1194-1210.Hecht, S. S., & Hoffmann, D. (1988). Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis, 9*, 875-884.Preston-Martin, S. (1987). N-nitroso compounds as a cause of human cancer. *IARC Scientific Publications, 84*, 477-484.Carmella, S. G., Akerkar, S., & Hecht, S. S. (1993). Metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers’ urine. *Cancer Research, 53*, 721-724.Carmella, S. G., Akerkar, S. A., Richie, J. P., Jr., & Hecht, S. S. (1995). Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers’ urine. *Cancer Epidemiology, Biomarkers & Prevention, 4*, 635-642.Carmella, S. G., Yoder, A., & Hecht, S. S. (2006). Combined analysis of r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in smokers’ plasma. *Cancer Epidemiology, Biomarkers & Prevention, 15*, 1490-1494.Xia, Y., McGuffey, J. E., Bhattacharyya, S., Sellergren, B., Yilmaz, E., Wang, L., & Bernert, J. T. (2005). Analysis of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in urine by extraction on a molecularly imprinted polymer column and liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Analytical Chemistry, 7*7, 7639-7645.Xia, Y., & Bernert, J. T. (in press). Stability of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine samples stored at various temperatures. *Journal of Analytical Toxicology*.Ashley, D. L., O’Connor, R. J., Bernert, J. T., Watson, C. H., Polzin, G. M., Jain, R. B., et al. (2010). Effect of differing levels of tobacco-specific nitrosamines in cigarette smoke on the levels of biomarkers in smokers. *Cancer Epidemiology, Biomarkers & Prevention*. doi: 10.1158/1055-9965.EPI-1-0084.Hecht, S. S., Carmella, S. G., Murphy, S. E., Riley, W. T., Le, C., Luo, X., Mooney, M., & Hatuskami, D. K. (2007). Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. *Cancer Epidemiology, Biomarkers & Prevention, 16*, 1567-1572.Hatsukami, D. K., Benowitz, N. L., Rennard, S. I., Oncken, C., & Hecht, S. S. (2006). Biomarkers to assess the utility of potential reduced exposure products. *Nicotine Tobacco Research, 4*, 600-622.Bernert, J. T., Pirkle, J. L., Xia, Y., Jain, R. B., Ashley D. L., & Sampson, E. J. (2010). Urine concentrations of a tobacco-specific nitrosamine carcinogen in the U.S. population from secondhand smoke exposure. *Cancer Epidemiology, Biomarkers & Prevention, 19*(11), 2969-77Xia, Y., Jain, R., Bernert, J. T., Ashley, D. L., & Pirkle, J. L. (2011). Tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers in the United States: NHANES 2007-2008. *Biomarkers, 16*(2), 112-119. |
| **Mode of Administration:** | Bioassay  |
| **Derived Variables:** | None |
| **Requirements:** |

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| **Requirement Category** | **Required (Yes/No)** |
| **Major equipment** | Yes |
| **Specialized training** | Yes |
| **Specialized requirements for biospecimen collection** | Yes |
| **Average time of greater than 15 minutes in an unaffected individual** | No |

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| **Annotations for Specific Conditions:** | None |
| **Process and Review:** | Not applicable.  |