About the Measure	
Domain:	Smoking Cessation
Measure:	Nicotine Metabolite Ratio – Serum and Saliva
Definition:	The ratio of nicotine metabolites trans-3' hydroxycotinine (3HC) / Cotinine (COT), a biomarker of CYP2A6 enzyme activity.
Purpose:	To measure the activity of the enzyme CYP2A6, which is the primary enzyme responsible for nicotine metabolism. A genetically-informed biomarker of nicotine clearance, the nicotine metabolite ratio (NMR) is associated with smoking cessation success and treatment response in clinical trials.
Essential PhenX Measures:	Current Age Cigarette Smoking Status - Adult
Related PhenX Measures:	Biomarker of exposure to nicotine-containing products – Saliva Biomarker of exposure to nicotine-containing products - Serum Current Environmental Tobacco Smoke Exposure Passive Smoke Exposure
Measure Release Date:	

About the Protocol	
Protocol Release Date:	
PhenX Protocol Name:	Nicotine Metabolite Ratio – Serum and Saliva
Keywords:	Biomarker, nicotine, CYP2A6, Cotinine, hydroxycotinine, serum, saliva, tobacco smoke exposure, tobacco product exposure, tobacco chemical exposure, smokeless tobacco exposure, laboratory protocol, Centers for Disease Control and Prevention, CDC, National Health and Nutrition Examination Survey, NHANES, laboratory protocol, Smoking Cessation
Protocol Name from Source:	National Health and Nutrition Examination Survey (NHANES) Cotinine and Hydroxycotinine Analysis

Nicotine Metabolite Ratio – Serum and Saliva **Date of SC final approval**

Description:	This is the laboratory protocol for measuring cotinine and trans-3'-hydroxycotinine in serum and saliva, which is used by the Centers for Disease Control and Prevention and for National Health and Nutrition Examination Survey (NHANES) cotinine analyses. The ratio of hydroxycotinine to cotinine, known as the Nicotine Metabolite Ratio (NMR), is correlated with the rate of nicotine metabolism in smokers and may be used to select the optimal type of smoking cessation medication.
Specific Instructions:	Cotinine (COT) and trans-3'-hydroxycotinine (HC) can be measured in serum, plasma, urine, and saliva. COT concentrations tend to be three to eight times higher in urine than in serum; however, plasma or serum is the fluid of choice for studies requiring a quantitative assessment of exposure. For that reason, serum was chosen as the matrix for the National Health and Nutrition Examination Survey (NHANES) COT analyses. In serum, HC concentrations tend to be two to four times lower than COT concentrations. This same method is used to measure COT and HC in saliva by substituting suitable saliva QC pools. All other aspects including calibration, cleanup and analysis are identical to serum procedures. Sample processing does not require anticoagulants, special preservatives, or unusual sterility procedures. However, the WG notes that plasma from anticoagulated blood can also be used. Blood can be collected from venipuncture by using standard equipment. Allow the blood to clot for a minimum of 30 minutes and up to 2 hours to create maximum serum yield. Transfer the serum to polypropylene cryogenic, screw-cap vials and freeze. Collection of saliva is most conveniently accomplished using a Salivette® or similar commercial device. Salivettes may be frozen directly after sample collection for subsequent transfer to the laboratory without any further processing required. The testing laboratory should be contacted before samples are collected to confirm the suitability of any equipment used to collect, process or store samples intended for these analyses. NMR data is commonly analyzed by quartiles, based on data for the particular study population. Typical NMR ranges and distributions vary by race and sex, and reference range information should be obtained as part of the individual research study being conducted. The Smoking Cessation Working Group recommends that the protocol can be used for adults aged 18 years and older who can provide a blood, saliva or urine sample.
Protocol:	Cotinine (COT) and trans-3'-hydroxycotinine (HC) are measured by an isotope-dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric (ID HPLC-APCI MS/MS) method. Briefly, the serum sample is spiked with methyl-D3-COT and methyl-D3-HC as internal standards. The sample is basified and then applied to a supported liquid extraction (SLE) plate. The analytes are extracted with an isopropanol/methylene chloride mixture, the organic extract is concentrated, and the residue is injected onto a C18 HPLC column. The eluent from these injections is monitored by APCI-MS/MS. The m/z 80 product ion from the m/z 177 quasi-molecular ion is measured for COT and the m/z 80 product ion from the m/z 193 quasi-molecular ion is measured for HC. Additional ions for the internal standards and for confirmation are also monitored for the respective

	compounds. Analyte concentrations are derived from the area ratios of native-to-labeled compounds in the sample by comparisons to a standard curve. The limit of detection (LOD) is 15 pg/ml. The nicotine metabolite ratio is then calculated as the ratio of HC to COT.
Selection Rationale:	Cotinine (COT) and trans-3'-hydroxycotinine (HC) are the primary metabolites of nicotine. The concentration of COT in body fluids can be used alone or in tandem with HC as a marker for active smoking. Because their concentrations are greater and their elimination half-lives significantly longer, these metabolites are generally preferred over nicotine itself as biomarkers. The ratio of HC to COT is called the nicotine metabolite ratio (NMR). It is highly correlated with the rate of nicotine metabolism in smokers. It is believed that the severity of nicotine dependence is related to an individual's rate of nicotine metabolism – the higher the NMR, the faster the metabolism of nicotine and the more dependent on nicotine the individual is. The conversion of nicotine to COT, as well as the conversion of COT to HC is largely mediated by the liver enzyme cytochrome P450 2A6 (CYP2A6). Thus, the NMR provides a convenient measure to phenotype individuals for CYP2A6 activity. CYP2A6 is also responsible for metabolic activation of carcinogenic tobacco-specific nitrosamines. Therefore, the NMR can be helpful in the development of individual pharmacotherapies for nicotine dependence (CDC, 2018).
Source:	CDC Laboratory Procedure Manual for Cotinine and Hydroxycotinine in Serum and Saliva, Laboratory Procedures Manual (2018)
Availability:	Publicly available
Life Stage:	Unavailable
Language:	English
Participant:	Unavailable
Personnel and Training Required:	Laboratory training for sample preparation, sample handling, and in the use of liquid chromatography and tandem mass spectrometry is required.
Equipment Needs:	This method requires high-performance liquid chromatography and tandem mass spectrometry.
General References:	Lerman, C., Schnoll, R. A., Hawk, L. W., Jr, Cinciripini, P., George, T. P., Wileyto, E. P., Swan, G. E., Benowitz, N. L., Heitjan, D. F., Tyndale, R. F., & PGRN-PNAT Research Group (2015). Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomized, double-blind placebo-controlled trial. *The Lancet. Respiratory medicine*, *3*(2), 131–138.

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<tr><td></td><td>Dempsey, D., Tutka, P., Jacob III, P., Allen, F., Schoedel, K., Tyndale, R. F., & Benowitz, N. L. (2004) Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther, 76, 64-72

Carroll, D. M., Murphy, S. E., Benowitz, N. L. Strasser, A. A., Kotlyar, M., Hecht, S. S., Carmella, S. G., McClernon, F. J., Pacek, L. R., Dermody, S. S., Vandrey, R. G., Donny, E. C., & Hatsukami, D. K. (2020) Relationships between the Nicotine Metabolite Ratio and a panel of exposure and effect biomarkers: Findings from two studies of U.S. commercial cigarette smokers. Cancer Epidemiol Biomarkers Prev. (in press)

St.Helen, G., Novalen, M., Heitjan, D. F., Dempsey, D., Jacob III, P., Aziziyeh, A., Wing, V. C., George, T. P., Tyndale, R. F., & Benowitz, N. L. (2012) Reproducibility of the Nicotine Metabolite Ratio in cigarette smokers. Cancer Epidemiol Biomarkers Prev, 21(7), 1105–14

Park, S. L., Tiirikainen, M. I., Patel, Y. M., Wilkens, L. R., Stram, D. O., Le Marchand, L., and Murphy, S. E. (2016) Genetic determinants of CYP2A6 activity across racial/ethnic groups with different risks of lung cancer and effect on their smoking intensity. Carcinogenesis, 37(3), 269-279</td></tr>
<tr><td>Mode of Administration:</td><td>Bioassay</td></tr>
<tr><td>Derived Variables:</td><td>None</td></tr>
<tr><td>Requirements:</td><td>
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<tr><th>Requirements Category</th><th>Required (Yes/No):</th></tr>
<tr><td>Major equipment</td><td>Yes</td></tr>
<tr><td>Specialized training</td><td>Yes</td></tr>
<tr><td>Specialized requirements for biospecimen collection</td><td>No</td></tr>
<tr><td>Average time of greater than 15 minutes in an unaffected individual</td><td>No</td></tr>
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<tr><td>Annotations for Specific Conditions:</td><td>Major equipment: Automated sample preparation system, liquid handler, High Performance Liquid Chromatography (HPLC), mass spectrometer

Specialized training: training for sample preparation, sample handling, and instrument operation is required</td></tr>
<tr><td>Process and Review:</td><td>Not Applicable</td></tr>
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