Development of an Assay for Urine based biomarkers to detect Breast Cancer.

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It is estimated that there will be 209,060 new cases of breast cancer diagnosed and 40,230 deaths from breast cancer in 2010. Currently, a woman has a 1 in 8 chance of developing this disease in her lifetime (SEER). Diagnosis is expensive, invasive and all too often - late. Early detection is usually vital in order for treatment to be successful. A new and more sensitive approach to breast cancer diagnosis might be found in metabolomics. Metabolomics takes into consideration the changes in small molecules, metabolites, produced from the many cellular reactions within the body. Various biofluids have been used for metabolic profiling but due to its ready availability and ease of acquisition, urine is an ideal choice for this type of analysis. The present study will use NMR analysis of urine from subjects showing no signs of disease and from those with a positive diagnosis encompassing all stages of breast cancer. From these urine samples, specific metabolites will be identified using 2D NOESY on a 400MHz Varian NMR. The resulting data will be processed using a Chenomx software package. A Umetrics statistical software program will then be used to further identify the statistically significant parts of the spectra. By finding metabolites in urine that are indicative of breast cancer, especially in the vital earliest stages, it will be possible to not only detect these early lesions but to also quickly provide the therapeutic regime with the best chance of eradicating them.

Before metabolomics data can be acquired operators must go through a certification protocol in order to minimize any systematic error as a function of operator and method. To begin this process a solution of sucrose was prepared to an exact concentration with D_2O. The sucrose sample was then analyzed multiple times by multiple operators using the 400MHz Varian NMR. The data from these spectra were then input into the Umetrics statistical software program, SIMPCA P+. Analysis of the individual operator spectra as well as analysis of multiple operators for consistent data analysis and acquisition was carried out to assure a confidence level of 95%. Following certification of operators with sucrose samples, sterile urine was obtained as an example for multivariate analysis. The sterile urine is spiked with an internal standard, and a pH indicator as well as sodium azide to prevent bacterial growth. Analysis of these urine samples is carried out using the same Chenomx, Umetrics protocols used for sucrose to obtain consistent spectral data acquisition as well as analysis. This Chenomx, package allows not only for predicted metabolites within the spectra to be identified through targeted profiling but also permits for a quantitative look at each possible metabolite. For the present protocol spectral binning was used to analyze the spectra in Chenomx. A principle component analysis (PCA) was then used to identify relevant portions of the spectrum. The protocols developed from this systematic analysis will be used on human urine samples to identify metabolic profiles for breast cancer.