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Dielectrophoretic device for separation of DNA, RNA, exosome biomarkers and drug delivery nanoparticles from whole blood, plasma and serum

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New dielectrophoretic (DEP) devices and techniques now allow rapid isolation and detection of circulating cell free (ccf) DNA, ccf-RNA, exosome biomarkers and drug delivery nanoparticles directly from un-diluted blood, plasma and serum samples. DEP is a well-known technique for separations of cells, nanoparticles and biomolecules. However, until recently, DEP was impractical for use with blood, plasma, serum and other high conductance solutions (>0.1 S/m) i.e., considerable sample dilution was required before DEP could be carried out. Using new DEP microarray devices ccf-DNA, ccf-RNA, exosomes and drug delivery nanoparticles can now be isolated in 10-20 minutes directly from small volumes (25-100 μ l) of blood, plasma or serum. Circulating cf-DNA isolated from chronic lymphocytic leukemia (CLL) patient blood and plasma samples by DEP were PCR amplified to identify the VHL genotype and then sequenced. Sequencing results for DEP were comparable to two gold standard methods. DEP was used to carry out the isolation of glioblastoma exosomes from 50 μ L of human plasma. RT-PCR analysis of ccf-RNA from exosomes showed presence of mRNA (beta-actin) and mRNA for cancer-specific EGFRvIII. Finally, the DEP was used for the recovery of drug delivery nanoparticles from undiluted human plasma samples. DEP was successful in isolating a wide range of drug delivery nanoparticles, including low density nano-liposomes. New DEP technology sets the stage for seamless sample to answer molecular diagnostics and therapy monitoring, which allows a variety of important cancer and other biomarkers and drug delivery nanoparticles to be rapidly isolated directly from whole blood and other clinical samples.

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Capillary zone electrophoresis as a tool for proteomics research

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Capillary zone electrophoresis-tandem mass spectrometry (CZE-MS/MS) has recently attracted attention as a tool for shotgun proteomics. However, its performance for this analysis has fallen far below that of reversed phase liquid chromatography (RPLC)-MS/MS. Here, we report the use of a CZE method with a wide separation window (up to 90 min) and high peak capacity (~ 300). This method is coupled to an Orbitrap fusion mass spectrometer via an electro-kinetically pumped sheath flow interface for analysis of complex proteome digests. Single-shot CZE-MS/MS identified over 10000 peptides and 2100 proteins from a HeLa cell proteome digest in ~ 100 min. This performance is nearly an order of magnitude superior to earlier CZE studies and is within a factor of 2 to 4 of state-of-the-art nano ultrahigh pressure LC system.

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