189 NANBIOTECHNOLOGY: DEVICES TO QUANTIFY CELLS AND THEIR FUNCTIONS.

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In vivo, the efficacy of an immune response is dependent on the existence of the appropriate types and numbers of immune cells and their ability to communicate. The ability of immune cells to properly communicate is dependent on their ability traffic to centers of interaction (secondary lymphoid organs) and effector sites (sites of infection or cancer) in tissues. Cell trafficking requires expression of chemokine receptors and adhesion molecules as well as cytoskeletal changes affecting cell deformability. A microelectronic device consisting of a 100 μm^2 electrode coated with antibody to capture specific cell types has been developed to quantify the absolute number of a lymphoid subset in one µL of blood. Three additional microdevice designs have been developed to assess cell deformability and chemotaxis. Differential deformability and chemotaxis occurs after various forms of stressors (e.g., oxidative stress and cold restraint), which cause a loss of reactive cellular thiols. Differences in deformability and chemotaxis have been observed with blood leukocytes from young (<6 mon) vs older (>18 mon) mice as well as with blood leukocytes after *in vitro* exposure to impermeant (copper:phenanthroline) or permeant (hydrogen peroxide) oxidants. In cold restraint stressed mice, the subset that appears most sensitive to loss of surface thiols is the NK population. Loss of surface thiols is shown to prevent lymphocytes from being able to flow through into 5 µm channels or chemotaxis through 5 µm gaps in response to SDF-1α or C3a. Supported by the Nanabiotechnology Center, a STC program of NSF (ECS-9876771).

190 A HEADSPACE PROCEDURE FOR THE QUANTIFICATION OF 1- AND 2-BROMOPROPANE IN HUMAN URINE.

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A test procedure was developed to detect and quantify the levels of 1- and 2-bromopropane in urine. 1-Bromopropane (1-BP) is a commonly used industrial solvent, and 2-bromopropane (2-BP) is often found as an impurity component in industrial grade 1-BP. Both are of a health concern for exposed workers because of their chronic toxicity. Central neurological and peripheral neuropathy disorders have been reported in workers exposed to 1-BP; reproductive and hematopoietic disorders has been reported for workers exposed to 2-BP. In the test procedure described, urine samples were diluted with deionized water and placed into a sealed headspace vial. A static-headspace sampler (Teledyne-Tekmar Model 7000) was used to heat each sample at 75oC for a 35 minute equilibrium time. Quantification of the two analytes was by means of a gas chromatograph equipped with a dimethylpolysiloxane capillary column and an electron capture detector. 1-Bromobutane was used as an internal standard for this test procedure. A multi-level recovery study using fortified urine samples (0.5 to 8 µg/ml) demonstrated full recovery; 104 to 121% recovery was obtained. Precision ranged from 5 to 17 % for the 15 to 20 spiked samples at each level analyzed over multiple experimental trial days. The limit of detection or this test procedure was approximately 2 ng/ml 1-BP and 7 ng/ml 2-BP in urine. A six week storage study was conducted to determine the most appropriate method to collect and store fortified urine samples. Glass serum vials with crimped caps and Teflon lined septa stored at 4oC demonstrated full recovery of both 1-BP and 2-BP.

191 EVALUATION OF TEST PROCEDURES FOR THE QUANTIFICATION OF URINARY (2-METHOXYETHOXY)ACETIC ACID.

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(2-Methoxyethoxy)acetic acid (MEAA) is a metabolite and biomarker for exposure to 2-(2-methoxyethoxy)ethanol [diethylene glycol monomethyl ether, DEGME, or DiEGME], a glycol ether which is of concern because of general toxicity. Glycol ethers have been frequently reported to damage the hemopoietic system, the male reproductive system and have demonstrated fetal/embryonic developmental toxicity. Specifically of interest to this laboratory is the use of 2-(2-methoxyethoxy)ethanol as an anti-icing additive to the military jet fuel JP-8. 2-(2-Methoxyethoxy)ethanol is readily absorbed through the skin and of concern for human dermal exposure. Test procedures to quantify the level of MEAA in human urine were developed and compared. Gas chromatography using a mass selective detector and a 50-m X 0.20-mm (id) dimethylpolysiloxane capillary column were used in each procedure studied. Two derivatization procedures were used in this evaluation. First, MEAA was extracted from fortified urine with ethyl acetate. Esterification of MEAA to the corresponding ethyl ester was one approach, and derivatization of MEAA to the corresponding t-butyldimethyl silane derivative was the second approach for gas chromatographic anaylsis. Recovery studies using 2, 5, 10 and 20 µg/ml MEAA fortified human urine samples demonstrated good accuracy and precision for both procedures. Recoveries using the ethyl ester procedure varied between 95-105% with precision [measured as percent relative standard deviation (%RSD)] as high as 14.3%, and recoveries using the silylation reagent were between 94-99% with precision (%RSD) as high as 7.3%. The t-butyldimethyl silane derivative procedure was less labor intensive and demonstrated better precision. The diethyl ester derivative procedure had better chromatographic performance from the more extensive sample cleanup.

192 AUTOMATIC BIOSENSOR ANALYZER FOR RAPID ASSAY OF NEUROPATHY TARGET ESTERASE (NTE) IN BLOOD.

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Previous studies showed that whole blood NTE measured with a biosensor is a reliable biomarker of exposure to neuropathic (NP) organophosphorus compounds (OPs). For rapid assessment of human exposure to NP OPs, a prototype electrochemical flow-injection device for analysis of NTE in blood was created. The device operates automatically with 96-well plates and allows 8 samples to be analyzed simultaneously. NTE is assayed by the differential inhibition method using phenyl valerate as substrate. All sampling and analysis steps are carried out and monitored automatically. A "user friendly" interface enables all functions easily to be controlled and monitored by a computer and specially developed software, so that a minimum of operator effort is required. Data processing and calculation of NTE activity are done automatically using a phenol calibration curve. A new approach to construction of the amperometric biosensors was studied using layer-by-layer technology, resulting in 4-fold enhancement of biosensor sensitivity. Validation of electrode measurements was done using hen brain NTE. NTE activities and I_{50} values for NTE inhibitors determined with the standard spectrophotometric method were statistically identical to respective values obtained by the new LBL biosensor. Using the new device, blood and brain NTE inhibition by mipafox, DFP, ethyl- and propyl-dichlorvinylphosphates, and three fluorinated 1-aminophosphonates, $(RO)_2P(O)C(CF_3)_2NHS(O)_2C_6H_5$, were well correlated ($r$ = 0.995, n = 7), supporting the use of blood NTE as a biochemical marker of exposure to NP OPs. The results indicate that the new device could be used for rapid assessment of human exposures to NP OPs, e.g., to support consequence management and minimize risks of chemical terrorist attacks. (Supported by CRDF RB2 2488 and ARO DAAD19-02-1-0388).

193 AN IMPROVED METHOD FOR THE QUANTITATION OF 8-HYDROXY-2'-DEOXYGUANOSINE IN BIOLOGICAL SAMPLES.

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8-Hydroxy-2'-deoxyguanosine (8-oxodG) is an important biomarker of oxidative stress. Numerous methods have been established for quantitation of 8-oxodG, including HPLC-ECD, LC-MS/MS, GC/MS, ELISA and the comet assay using FPG. However, concentrations several orders of magnitude different have been reported for endogenous 8-oxodG, demonstrating the need for an improved quantitation method. Moreover, 8-oxodG is prone to artifactual formation, which must be prevented to achieve accurate quantitation. We hypothesized that coupling immunoaffinity (IA) chromatography to capillary LC-ESI^+-MS/MS would provide the requisite accuracy, sensitivity, and specificity required to measure 8-oxodG in a variety of samples while using reasonable amounts of DNA. Capillary LC typically offers 10-fold greater sensitivity over micro LC; thus, we developed such a method for the analysis of 8-oxodG. The presence of the free radical scavenger 2, 2, 6, 6-tetramethylpiperidine-*N*-oxyl (TEMPO) during DNA isolation and enzymatic hydrolysis prevented artifactual formation of 8-oxodG. IA clean-up resulted in selective isolation of 8-oxodG from digested DNA samples and reduced the appearance of interference peaks compared to HPLC clean-up. 8-OxodG was quantified using stable isotope internal standard, [$^{15}N_5$]-8-oxodG. The limit of detection was determined with standards to be 0.5 fmol on column with a signal-to-noise ratio of 3, enabling the detection of 8-oxodG in as little as 5 µg DNA. Preliminary data

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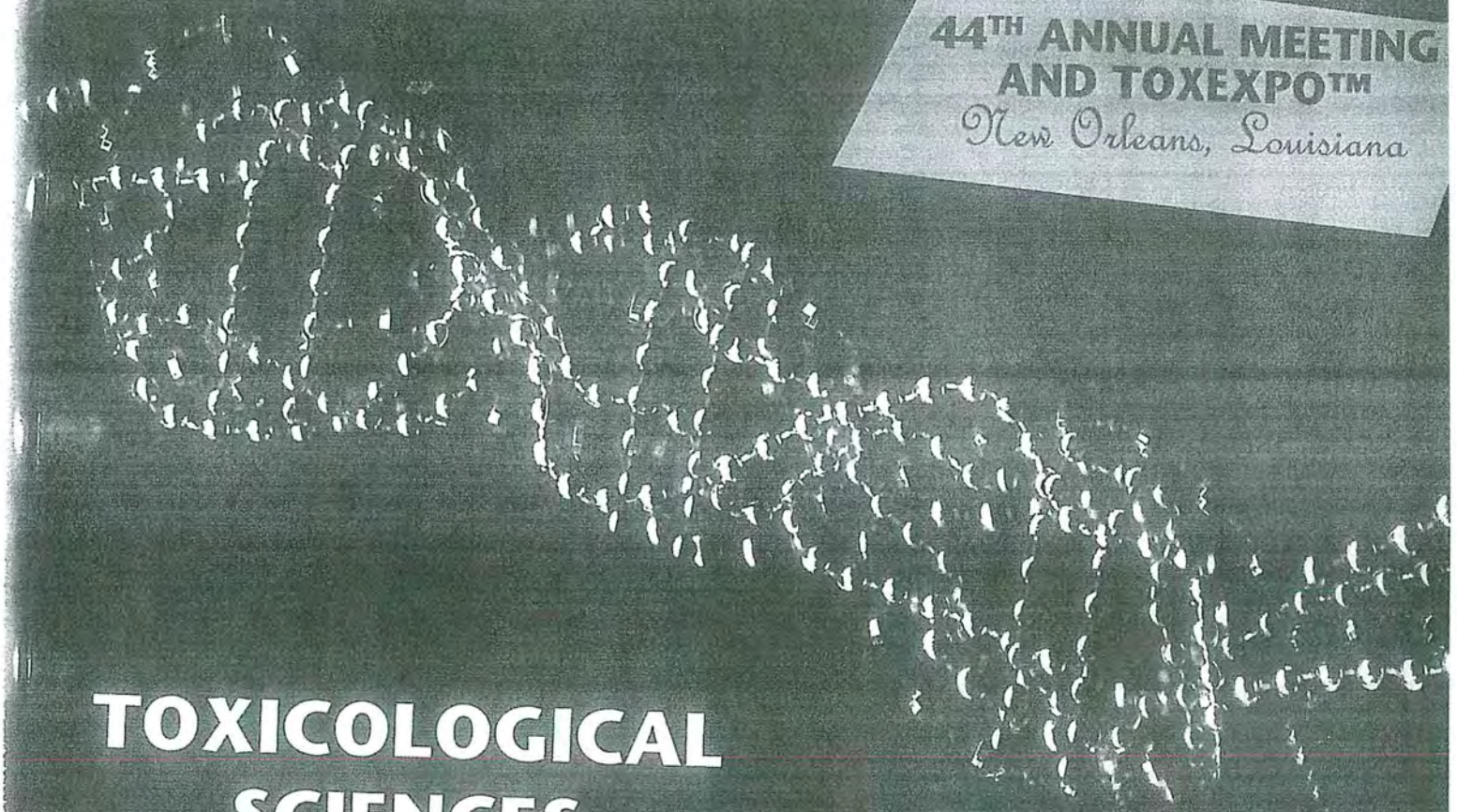

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