Project #1025388

Title: Field-Portable Immunoassay Instruments and Reagents to Measure Chelators and Mobile Forms of Uranium

Principal Investigator: Blake, Diane A.

Organization: Tulane University

Results To Date: Progress Report Date: 01/23/06 (report delayed due to Hurricane Katrina) Report of results to date: The goals of this 3-year project are to: 1) update and successfully deploy our present immunosensors at DOE sites; 2) devise immunosensor-based assays for Pb(II), Hg(II), chelators, and/or Cr(III) in surface and groundwater; and 3) develop new technologies in antibody engineering that will enhance this immunosensor program. Note: Work on this project was temporarily disrupted when Hurricane Katrina shut down the University on August 29, 2005. While most of the reagents stored in our refrigerators and freezers were destroyed, all of our hybridoma cell lines were saved because they had been stored in liquid nitrogen. We set up new tissue culture reactors with the hybridomas that synthesize the anti-uranium antibodies, and are purifying new monoclonal antibodies from these culture supernatants. Both the in-line and the field-portable sensor were rescued from our labs in New Orleans in early October, and we continued experiments with these sensors in the temporary laboratory we set up in Hammond, LA at Southeastern Louisiana University. We returned to our laboratories in New Orleans on January 3, 2006. Aim 1, Sensor updates and field testing: In February of 2005, the PI called a meeting with Sapidyne Instruments Inc. to discuss improvements to the immunosensors under development for this project. The in-line sensor was performing well and required only modest improvements in the hardware and software. These were implemented and an upgraded in-line instrument was delivered to the PI at Tulane in March of 2005. We have been using this sensor to test samples from the Criddle site at the FRC (ORNL). A paper describing the operation of the in-line sensor was published this year in Int. J. Environ. Anal. Chem. (see Publications, below). The field-portable sensor previously delivered by Sapidyne was inadequate, and during the February 2005 meeting Sapidyne's president agreed to completely redesign/rebuild this instrument. A new prototype was delivered to Tulane in May of 2005. Testing of the prototype progressed with both commercially available antibodies (to caffeine) and with the anti-uranium antibodies already isolated and characterized by our laboratory. A 2nd meeting, scheduled for September to finalize the design of the field-portable instrument, was delayed when Hurricane Katrina shut down the University. In January of 2006, the PI traveled to Sapidyne to provide this company with the data obtained using the prototype field-portable sensor. Based on these data, all parties agreed on the final design specifications for the new field-portable sensor. The CAD drawings for the instrument will be delivered to the PI in February, and the company will deliver the instrument in April or May. Sapidyne plans to make 2 instruments, one for our lab to test and a second to use at Sapidyne as needed for repairs or upgrades. We have recruited a new graduate student, Scott Melton, to work on the handheld instrument. Immunoassay studies during the first year have focused upon a comparison of our 3 anti-uranium antibodies, to determine which will work best in the groundwater at ORNL. Several groundwater samples from the Criddle site are being tested. At present, the monoclonal from hybridoma clone 12F6 appears to be most resistant to interferences from the groundwater matrix. Aim 2, Immunosensor-based assays for

Pb(II), Hg(II), chelators, and/or Cr(III) in surface and groundwater: In the first year of this project, we compared the ability of two commercially available instruments to accurately assess the binding properties of monoclonal antibodies; we found the KinExA 3000 Instrument to be superior to instruments that use surface plasmon resonance. When the binding properties of 2 the antibodies that we plan to use for these assays were analyzed, we discovered that these antibodies exhibited allosteric binding (positive cooperativity). Both of these studies were published in 2005 (see Publications, below). A new graduate student, Marcia Henry has been recruited to work on the antibody-based assays using these antibodies. Ms. Henry has an award from the Louisiana Board of Regents that pays her stipend. She was forced to evacuate to Houston in the aftermath of Katrina, but returned to our Tulane lab on January 12, 2006. The purified monoclonal antibodies she planned to use for these studies (2C12 and 2D42) were destroyed during the storm. The hybridomas survived, however, and her first task will be to set up tissue culture reactors to make more of these 2 antibodies. Aim 3, Development of new technologies in antibody engineering. A single chain antibody (scFv) directed toward a metal-chelate complex was isolated from the Human Synthetic VH + VL scFv phage-displayed antibody library provided to our lab by the Centre for Protein Engineering: Medical Research Council (Cambridge, England). Although this antibody bound with very low affinity, these experiments allowed us to develop techniques for library screening that will be useful when we have prepared our own scFv library using RNA from immunized animals.

Deliverables: Publications during this funding period (01/01/05-12/31/05): H. Yu, R.M. Jones, and D.A. Blake (2005) "An immunosensor for autonomous in-line detection of heavy metals: Validation for hexavalent uranium", Int. J. Env. Anal. Chem. 85:817-830. R.C. Blake II, N. Ohmura, S.J. Lackie, X. Li, J.B. Delehanty, I.A. Darwish, and D.A Blake (2005) "Monoclonal antibodies that exhibit allosteric binding behavior", In Monoclonal Antibodies: New Research, (M.A. Simons, ed.) Nova Science Publishers, Inc., Hauppauge, New York, pp 1-33. R.C. Blake II and D.A. Blake (2005) "Quantitative analysis of antibody-antigen interactions using immobilized ligands: Kinetic exclusion assays are more accurate than surface plasmon resonance", Progress in Monoclonal Antibody Research, (M.A. Simons, ed.) Nova Science Inc., Hauppauge, New York, pp 109-135. A.M. Kriegel and D.A. Blake (2005) "Antibody fragments with specificity for metal-chelate complexes from a human semi-synthetic phage display library", Recent Research Developments in Bioconjugate Chemistry, 2:127-143. Presentations during this funding period (01/01/05-12/31/05): D.A. Blake, H. Yu., R.C. Blake II, X. Li, I.A. Darwish, and M.B. Henry (2005) "Antibodies to metal-chelate complexes: Binding properties and applications in the life sciences", Gordon Research Conference on the Cell Biology of Metals, Lewiston, ME, July 3-8. X. Li, A.M. Kriegel, T.C. Bishop, R.C. Blake II, E. Figueiredo, H. Yu, and D.A. Blake (2005) "A recombinant antibody that binds chelated uranyl ions", Gordon Research Conference on the Cell Biology of Metals, Lewiston, ME, July 3-8. D.A. Blake, H. Yu, and R.C. Blake (2005) "Antibodies and Immunosensors for the Detection of Hexavalent Uranium", Society for Industrial Microbiology, Chicago, IL, August 21-25.