FIELD-PORTABLE AND AUTOMATED IMMUNOSENSORS FOR HEXAVALENT URANIUM, OTHER HEAVY METALS AND CHELATORS

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The goal of this project is to continue the development of new techniques for the rapid, automated identification of radionuclides, metals and chelators that may contaminate surface and groundwater at DOE sites. A set of high-affinity, highly selective binding reagents (monoclonal antibodies) is being developed and refined to facilitate their use in two different immunosensors that can rapidly and accurately measure environmental contaminants in the field. Sensor technologies already in place will be the foundation for the development of additional assays for metal, radionuclide, and chelator contaminants. The research objectives for this project and progress to date are as follows:

1) To successfully deploy present immunosensors at DOE sites, by adapting the immunoassay procedures to site-specific geochemistries. We have tested our automated immunosensor for the ability to accurately measure hexavalent uranium at biostimulated sites at the Oak Ridge Field Research Center. Sensor analysis using one monoclonal antibody (clone #8A11) provided uranium values that were only 60% of that determined by ICP-MS; however, when we changed the identity of the monoclonal antibody (clone #12F6) used in the sensor, uranium values were 80-95% of that determined by ICP-MS. Calcium was identified as the matrix component that led to low uranium recoveries when the 8A11 antibody was used for the analysis.

2) To devise immunosensor-based assays for Pb(II), Hg(II), and/or Cr(III) in surface and/or groundwater. Sensor-based assays have been formulated for Pb(II) using existing antibodies in purified water amended with buffers and spiked with Pb(II). Activities in the coming year will concentrate on adapting these assays for analysis of surface and groundwater.

3) To develop new technologies in antibody engineering that will enhance the immunosensor project. The monoclonal antibody used in the uranium immunosensor, clone #12F6, has been expressed as a recombinant protein. Molecular modeling and subsequent site-directed mutagenesis of selected amino acid side chains in the binding site have led to a new protein that binds to uranium with ~50% higher affinity than the original antibody. A library of phage-displayed antibodies was prepared using gene fragments derived from immunized rabbits and sheep. An antibody fragment that binds to chelated U(VI) with micromolar affinity has been selected from this library.

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