Cost-Effective Monitoring of Environmental Chemicals in Breast Milk

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Introduction:
Human milk provides excellent nutrition for infants, yet environmental chemical contaminants raise concern for toxicity. This formative research seeks to develop and assess a reliable cost-effective method for screening of environmental chemicals in breast milk to facilitate large-scale research on child health and development.

Aims: 1) Develop and evaluate rapid, low-cost enzyme-based immunosorbent assay (ELISA) for near real-time analysis of breast milk for target chemicals, with comparison to gold-standard GC/MS; 2) evaluate performance and cost-effectiveness of the ELISA in fresh breast milk; and 3) assess storage stability of the chemicals in frozen breast milk.

Methods:
Phase I: Develop ELISA methods and train field staff in ELISA testing of fresh human milk. Initial chemicals include water-soluble atrazine (herbicide) and fat-soluble pyrethroids (insecticides). Phase II: Enroll 120 postpartum lactating mothers (60 per site). Assess clinical data and environmental exposures by maternal questionnaires. Collect breast milk samples at 1, 2, and 4 months postpartum. Follow on-site ELISA with transfer of samples to Battelle for GC/MS confirmation. Assess long-term stability of chemicals in frozen portions by GC/MS.

Results: Phase I ELISA development and training for atrazine is complete and for complex pyrethroids is ongoing. Figures 1, 2, and 3 demonstrate ELISA methods. Phase II, initiated with OMB-exempt enrollees (*rounded to zero), will proceed with full enrollment after OMB approval.

Conclusions: It is anticipated that ELISA screening will be sensitive and selective for detecting targeted chemicals in fresh human milk and also more cost-effective than conventional analytical methods for large-scale human environmental exposure monitoring.

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Figure 1. Battelle's ELISA Method

ELISA Method for the Detection of Atrazine in Human Milk

The preparation of the human milk samples for post analysis of atrazine in each sample is simple. An aliquot of fresh human milk (1 mL) is mixed with HPLC-grade reagent water in a 1:2 proportion. This solution is then gently mixed and filtered through a syringe packed with glass wool.

Following this sample preparation, the human milk samples are tested for the presence of atrazine employing an enzyme-linked immunosorbent assay (ELISA). After the sample has been introduced to the kit, (Abraxis Atrazine Assay Kit 100T, Abraxis LLC, Warminster, PA) an atrazine enzyme conjugate solution is added, followed by addition of a solution containing triazine-specific antibodies attached to paramagnetic particles. In a subsequent incubation period, the atrazine in the sample and the atrazine enzyme conjugate compete for positions on the antibodies in a ratio relative to their concentrations.

At the end of this reaction period, a magnetic field is applied evenly to the samples. After washing unbound reagents from the samples, a solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine is added. The enzyme-labeled atrazine bound to the atrazine antibody reacts with this solution creating a colored product. After an incubation period, the reaction is stopped by the addition of a diluted acid. The color developed is inversely proportional to the concentration of atrazine in the sample. Confirmation of the ELISA results is conducted by gas chromatography mass spectrometry (GCMS) in a multi-residual analysis.

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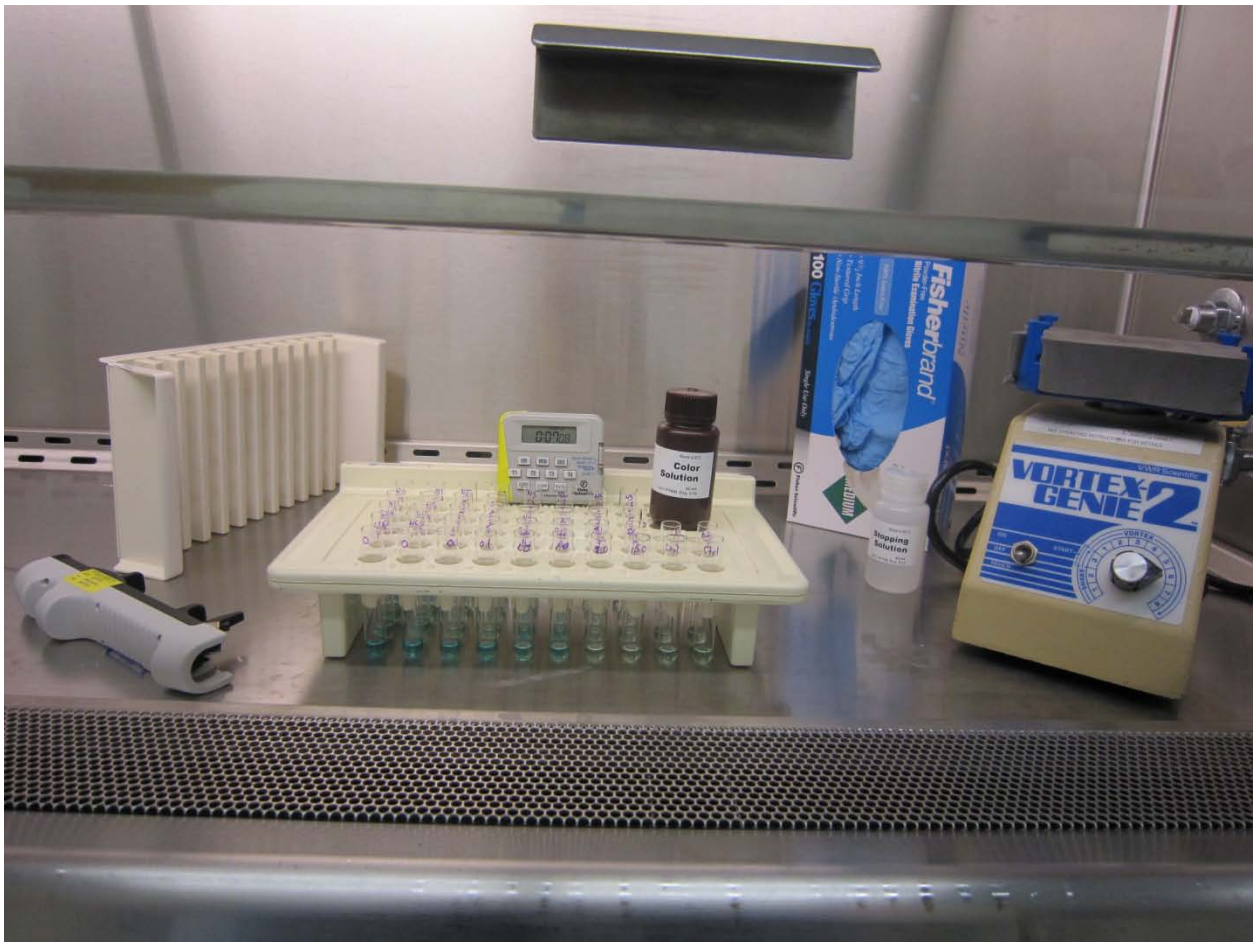
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