



***Using Biosensor
Technology for the
Long-Term Monitoring
of Groundwater for
Military-Unique
Compounds***

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USACE – ERDC – EL

Environmental Chemistry Branch

US Army Engineer Research & Development Center



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LTM Project

- **Long-term monitoring of groundwater at contaminated and/or remediated sites requires collection, transport, and analysis of samples for up to 30 years.**
 - **Costs associated with LTM using current techniques (billions)**
 - **Time associated with analysis, from collection to data report (weeks)**
 - **Generation of significant amounts of waste material (hazardous?)**

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LTM Project

- **Field analysis of sites for compounds of interest could significantly alleviate some of the issues facing LTM projects.**
 - **Lack of sensitive instruments and/or technologies for detection of military unique compounds (MUCs)**
 - **Quality of field-obtained data (screening)**



LTM Project

The mission of the LTM Groundwater Project of the Engineering Research and Development Center (ERDC) of the US Army Corps of Engineers is to develop effective field analytical technologies that are acceptable to all levels of the regulatory community and also meet the stewardship and fiscal needs of the Army.

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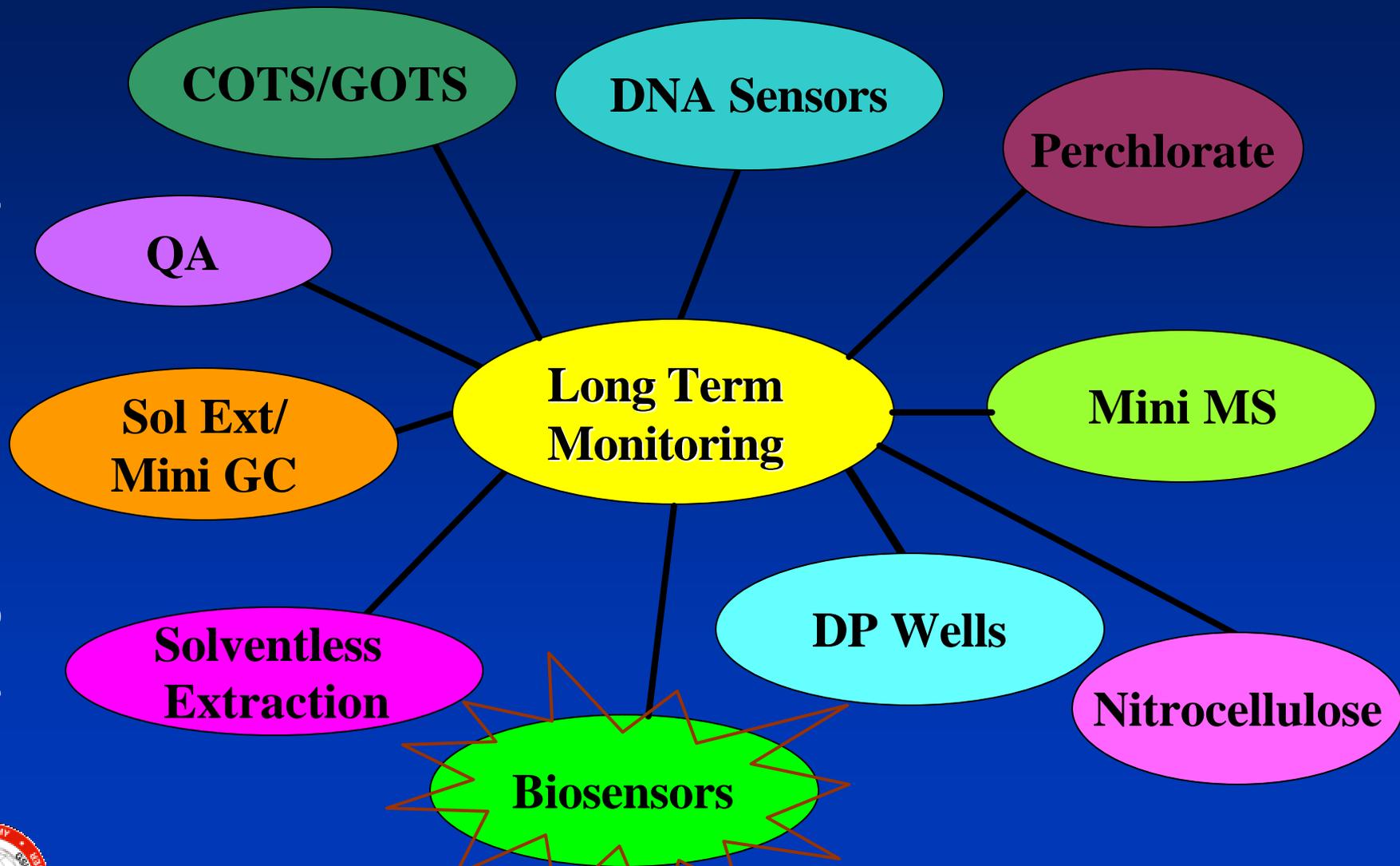
LTM Project

- **Technologies that are being developed within the LTM project will address the following requirements:**
 - **Quick analytical turnaround time (<4hrs)**
 - **Cost reductions of 25-50% compared to traditional laboratory analysis**
 - **Portability, Remote and *In Situ* Operation**
 - **Detection of MUCs at levels of concern**
 - **Defensible data generation**
 - **Acceptability to federal, state and local regulatory agencies**





Long Term Monitoring Focus Area



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LTM - Biosensors

Objective: Develop field-portable and/or field-deployable biosensors for the detection of MUCs in groundwater.

- **Biological-based versus Analytical-based detection systems**
- **Whole organism biosensors (screening)**
- **Capture Molecules**
 - **Antibodies**
 - **DNA (stay for next talk!)**





LTM - Biosensors

- **Immuno-based technologies (Antibodies)**
 - **Rapid (minutes)**
 - **Sensitive (ppb or ppt)**
 - **Specific**
 - **Small**
 - **Flexible / Adaptable**
- **Three primary techniques of immunoassays**
 - **Sandwich**
 - **Displacement**
 - **Competitive**
- **Fluorescent-linked detection**





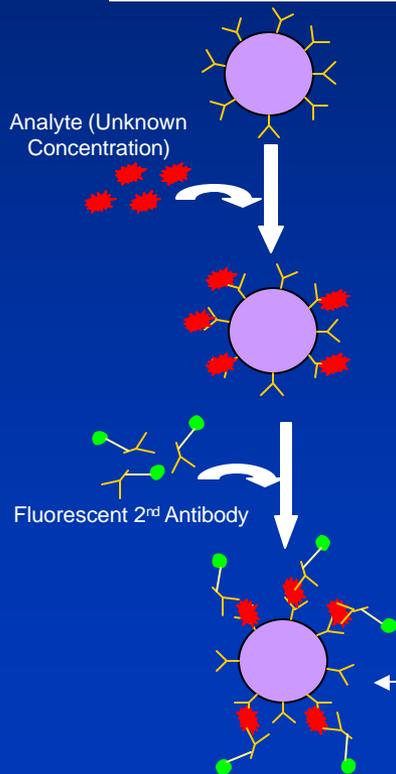
LTM - Biosensors

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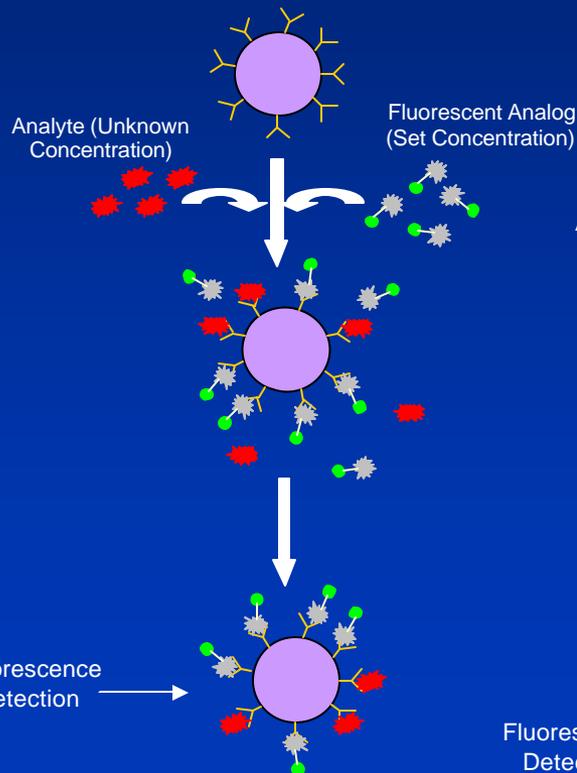
Y = Antibody to Analyte
● = Antibody Immobilized on Bead

● = Analyte
● = Fluorescent Analog
Y = Fluorescent Antibody

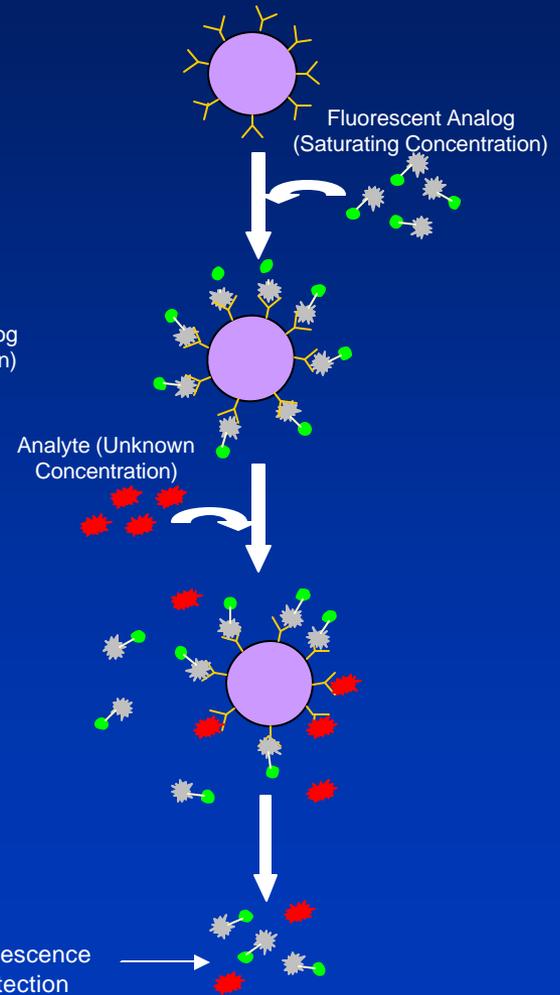
Sandwich Assay



Competition Assay



Displacement Assay





LTM - Biosensors

- **Conduct immunoassays (displacement and/or competition) utilizing antibodies immobilized on magnetic beads**

- **Expand the number of antibodies to MUCs**
 - **Antibodies to RDX and TNT are commercially available**
 - **Developing antibodies to HMX and 2, 4-DNT (SBS)**

- **Collaborate with other laboratories currently developing immuno-based technologies to integrate developed antibodies into the assay systems**

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LTM - Biosensors

- **Magnetic bead experiments**
 - RDX and TNT antibodies are commercially available (Strategic Biosolutions)
 - Immobilize antibodies onto surface of beads
 - Produce fluorescently-tagged analogs

- **Immunoassays with magnetic beads**
 - Competition
 - Displacement (Reverse Displacement)

- **Integrate beads into assay systems for field deployment**





LTM - Biosensors

- Conduct immunoassays (displacement and/or competition) utilizing antibodies immobilized on magnetic beads
- **Expand the number of antibodies to MUCs**
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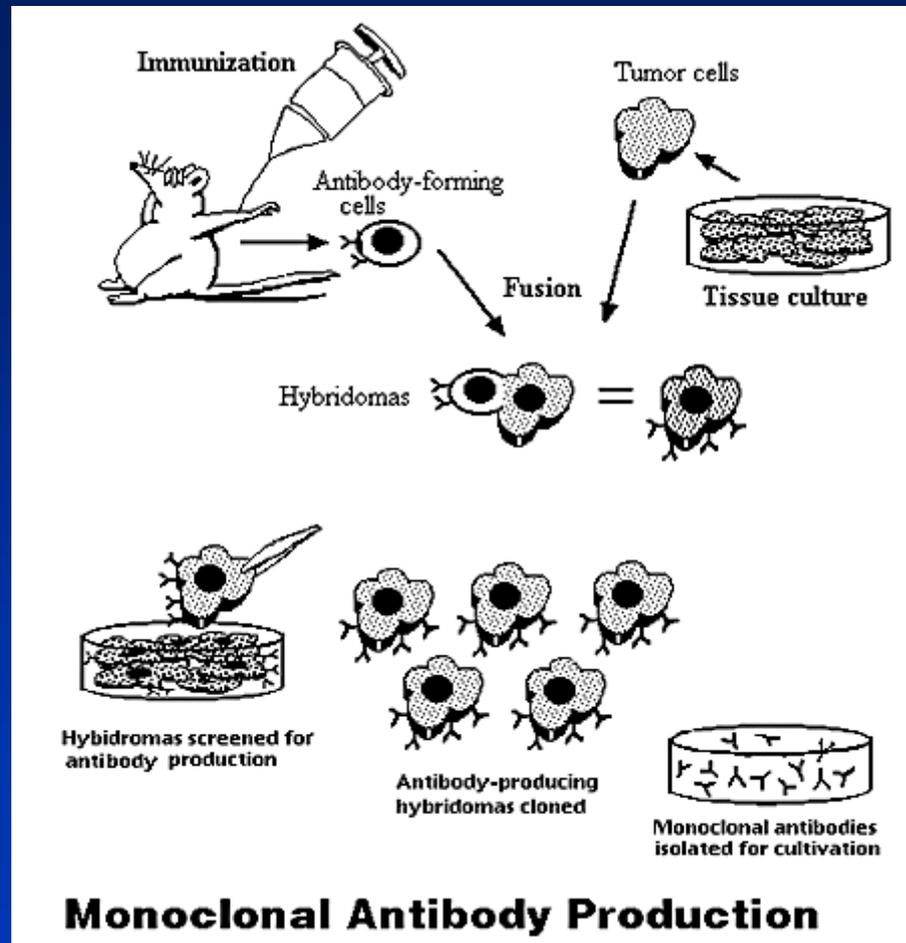
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LTM - Biosensors

Hybridoma Development (Strategic Biosolutions)

- Analytes
 - HMX
 - 2,4 - DNT
- Time Frame
 - 5 to 9 months per analyte



Access Excellence @ National Health Museum, 3-02-04
<http://www.accessexcellence.org/AB/GG/monoclonal.html>





LTM - Biosensors

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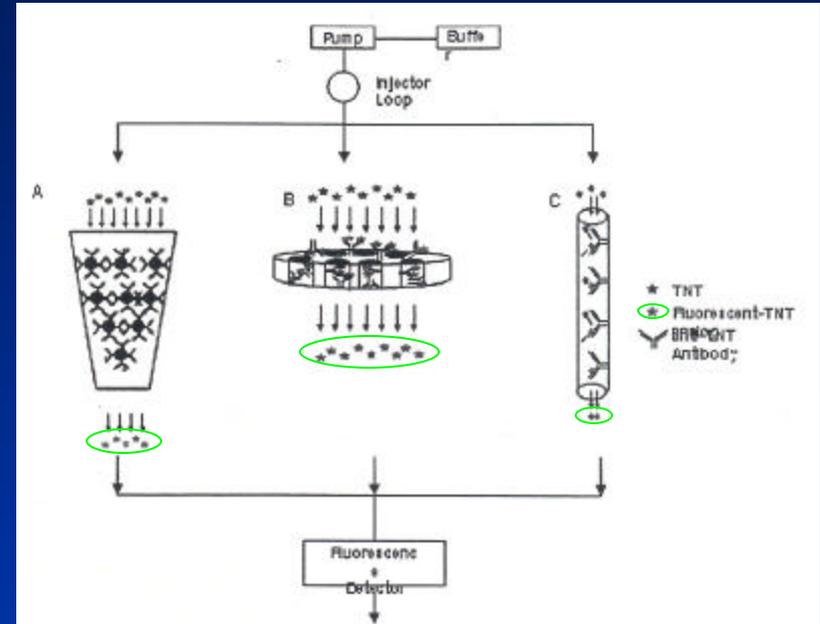




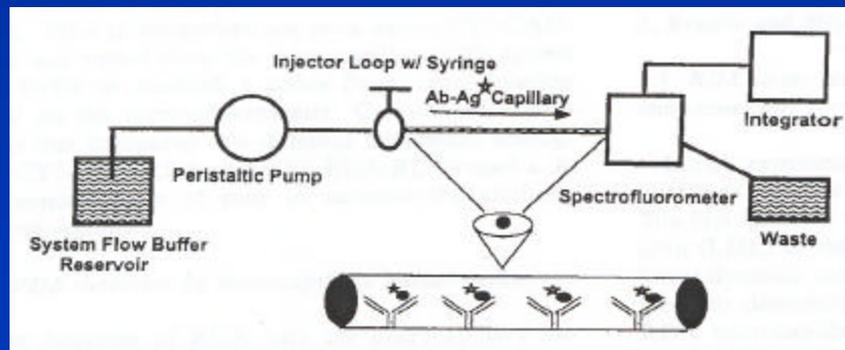
LTM - Biosensors

Continuous Flow Immunoassay

- Developed at Naval Research Laboratory
- Field-portable Displacement Immunoassay System
 - Bead-based Micro-Column (A)
 - Micro-porous Membrane (B)
 - Glass Micro-Capillary (C)



Shriver-Lake et al. (2003) *Anal Bioanal Chem*



Charles & Kusterbeck (1999)
Biosensors & Bioelectronics





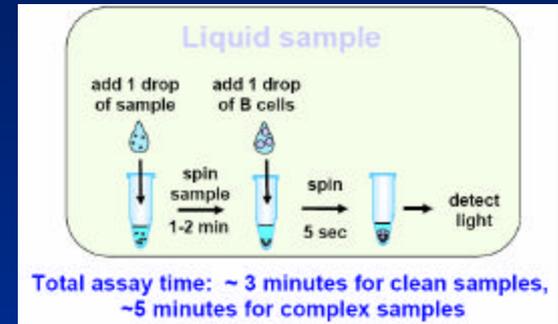
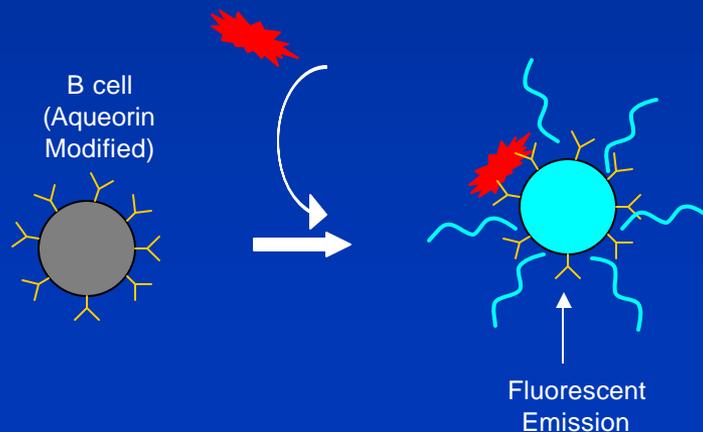
LTM - Biosensors

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CANARY (Cellular Analysis and Notification of Antigen Risk and Yields)

- Developed at MIT-LL
- Excellent for Biological Agents
 - *Bacillus anthracis* (anthrax)
 - *Yersinia pestis* (plague)
 - FMD (Foot and Mouth Disease) virus
 - *E. coli*
- Highly sensitive response in seconds
- Detection of Toxins (Small Molecules)
 - Developmental Stage

CANARY Bioassay



Lightweight COTS Components



Battery-powered operation > 6 hours
 Luminometer dimensions: 7" x 4" x 5 1/2"
 Total weight < 4 pounds including batteries

Source: Presentation at Federal Bio-Chem Detection Conference, Oct. 2003
 by Peter Emanuel, PhD, Critical Reagents Program Director, JPE-CBD

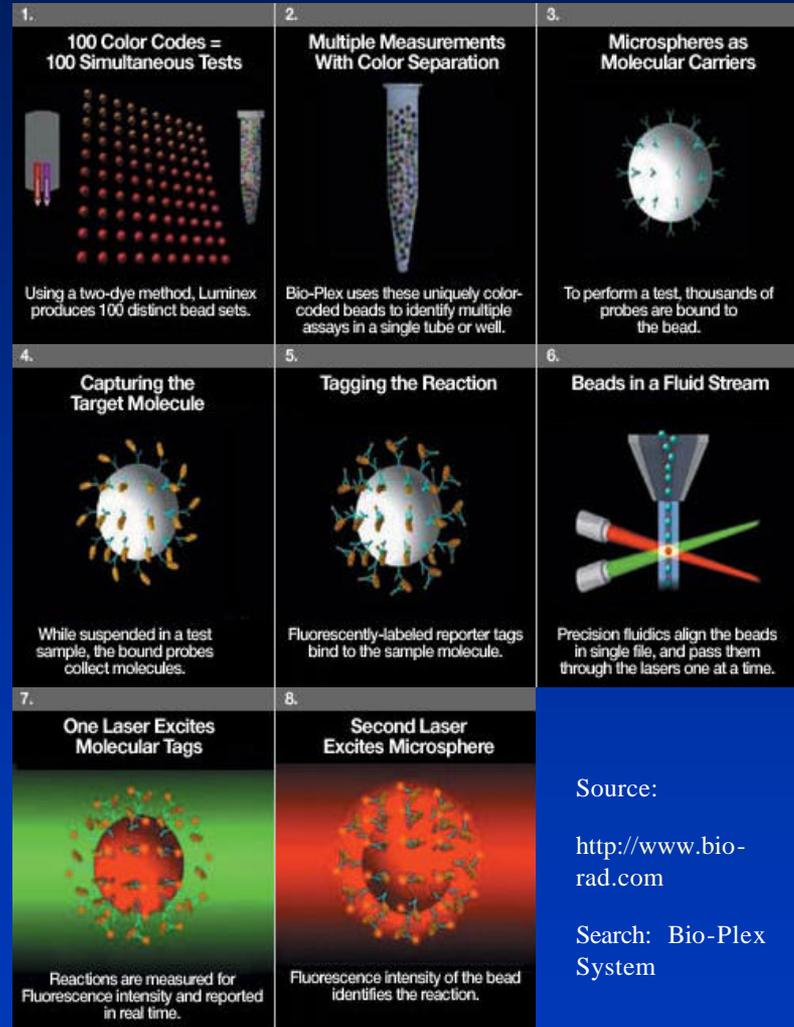




LTM - Biosensors

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- Luminex[®] xMAP[™] (Multi-Analyte Profiling) Technology
 - 100 unique analytes per assay
 - Minimal sample requirements (12 μ l)
 - Capture molecules include enzyme substrates, DNA, receptors, antigens or antibodies



Source:

<http://www.bio-rad.com>

Search: Bio-Plex System





LTM - Biosensors

- **Issues facing development of antibody-based biosensors...**
 - **Availability of Analogs for Analytes of Interest (Ab production & Detection Assays)**
 - **Production of Antibodies to Small Molecules (Daltons vs kDaltons)**
 - **Suitability of Method/Design for Detection of Small Molecules**

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LTM Project

- **Immuno-based biosensors address the requirements of the Long-Term Groundwater Monitoring Program**
 - **Reduced Analytical Times**
 - **Reduced Waste Production**
 - **Reduced Costs**
 - **Portable (potential exists for Remote, *In Situ* Operation)**
 - **Detection at Low Levels**
 - **Defensible Data Generation (quantifiable)**

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LTM Project

Mission of Long-Term Groundwater Monitoring Project is to develop efficient field analytical technologies.

- Reduce both cost and time of analysis
 - Portable, Remote and *In Situ* Operation
 - Detection of MUCs at levels of concern
 - Defensible data generation
-

Ultimately, the field technologies developed need to produce data that are acceptable to federal, state and local regulatory agencies.



LTM Project

Acknowledgements

LTM Project Manager

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Biosensors

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