US Army Engineer Research & Development Center

Long-Term Monitoring of Groundwater for Military Unique Compounds

Development of Biosensors

Dr. Shana R. Dalton, PhD
USACE – ERDC – EL
Environmental Chemistry Branch

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?)

LTM Project

- Field analysis of sites for compounds of interest could significantly alleviate some of the issues facing LTM projects.
  - Expense of current field methods
  - 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.

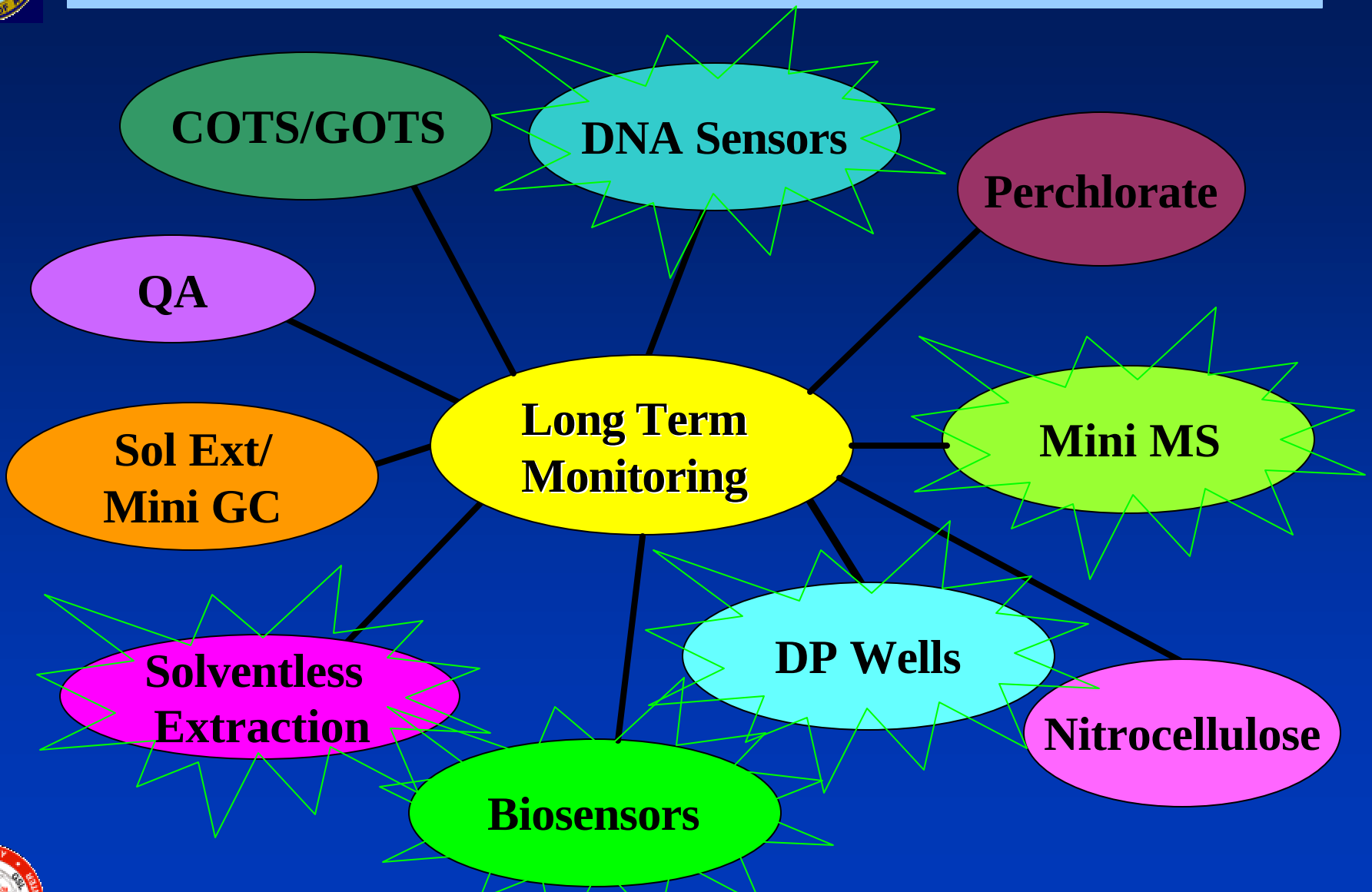
LTM Project

- The development and implementation/deployment goals of the project are being addressed in three major work areas.
  - New protocols for acquiring definitive data inside and outside of analytical laboratories
  - Currently available commercial and governmental technologies (COTS/GOTS)
  - New and emerging technologies for a real-time *in situ* monitoring system (RTISMS) for detection of volatile organics and MUCs

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 – Solventless Extraction

US Army Engineer Research & Development Center

Personnel: Dave Splichal & John Shannon

Objective - Develop and deploy *in situ* and/or field solventless extraction technologies with emphasis on military unique compounds (volatile organics and explosives).

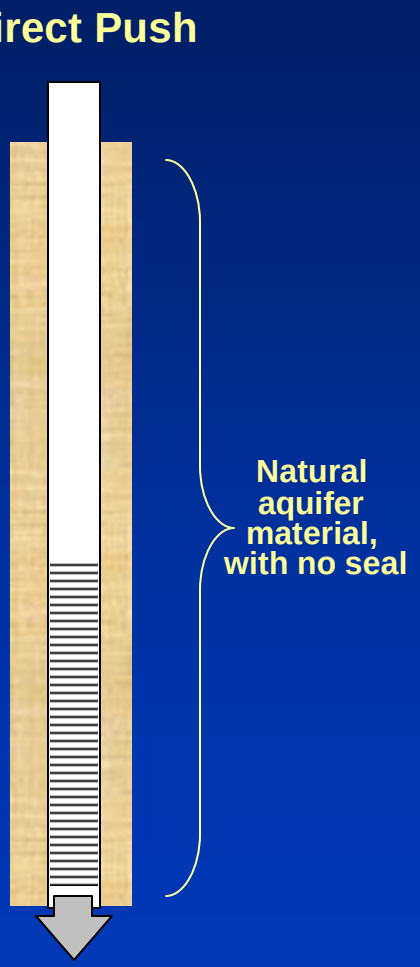
- **Technique 1: Twister – various coating capture analyte (Gerstel)**
- **Technique 2: Solid Phase Microextraction (SPME) – various coating capture analyte (Supelco)**
- **Technique 3: *In situ* extraction – modified Hydrasleeve (prototype built by EnoCon, Inc. and ERDC-EL-ECB)**

LTM – Direct Push Wells

US Army Engineer Research & Development Center

Personnel: Louise Parker

- **Advantages of DP wells**
  - **Quicker to install**
    e.g. At CRREL, it took 60 min to push 140 ft in glacial till
  - **No hazardous materials brought to surface**
  - **No drilling wastes that require disposal**
  - **Less expensive to install**
- **Concerns with low-flow purging and sampling procedure**
  - **By definition, sampling from the most permeable layer of the formation**
  - **Causes mixing in well and well bore**
  - **Time consuming**
  - **Expensive for dedicated equipment**
  - **Sorption of analytes by pump materials (esp. tubing)**



LTM – Direct Push Wells

US Army Engineer Research & Development Center

- Hydrasleeve Sampler (Cylindrical PE bag with PE check valve)

- Redesigned Hydrasleeve

- Diffusion Sampler

Don Vroblesky (USGS)

- Snap Sampler

S. Britt

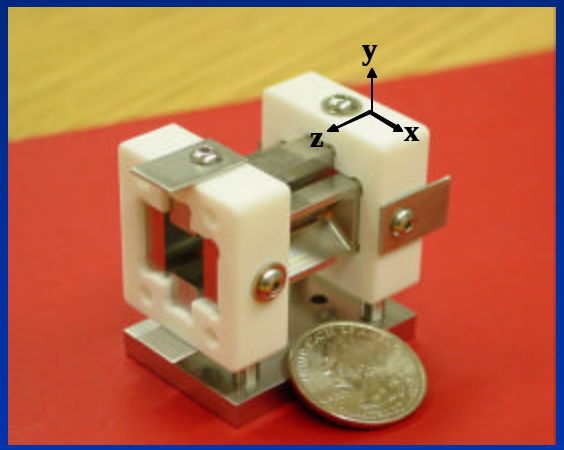
LTM – Mini MS

US Army Engineer Research & Development Center

Personnel: Denise MacMillan & Graham Cooks

Regulatory agencies will often not accept field analytical data without laboratory confirmation because…

- **Detection limits are above action levels**
- **Tests are often class rather than compound specific**
- **High potential for false positives**
- **Accuracy and QC issues**



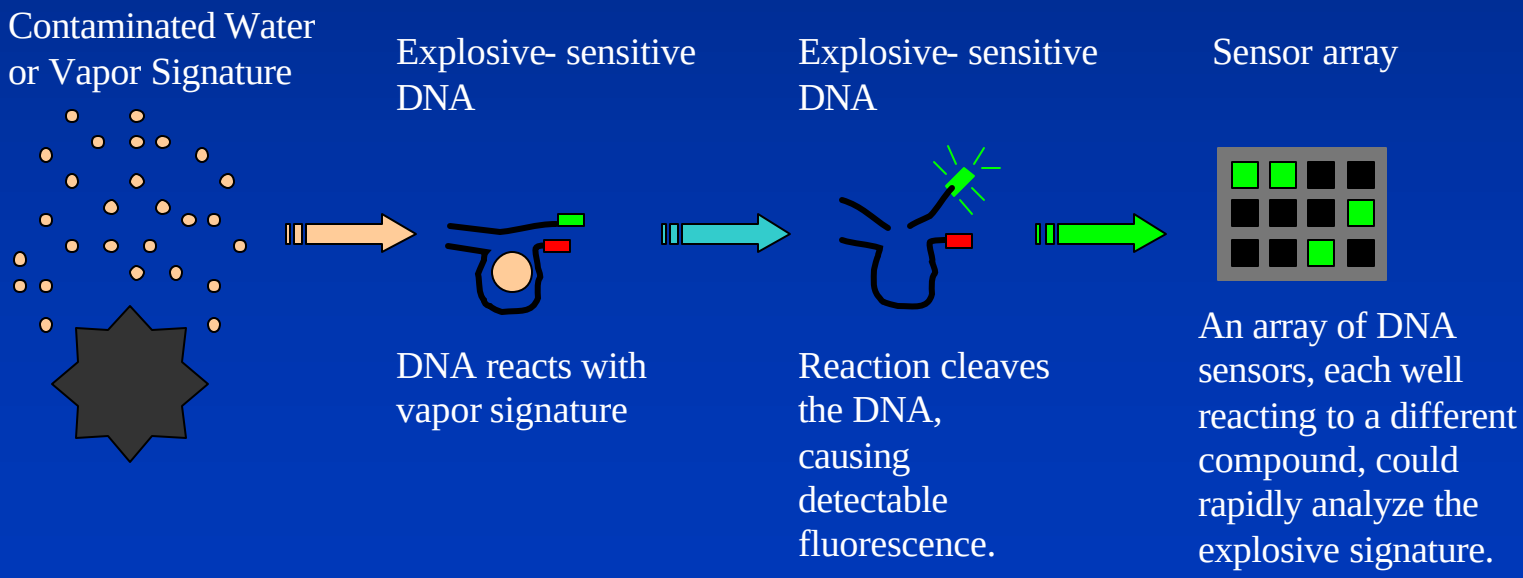
The ability to take a mass spectrometer into the field to conduct analysis would result in field data of a quality that is acceptable to regulatory agencies.

LTM – Catalytic DNA Sensors

US Army Engineer Research & Development Center

Personnel: Don Cropek

- **Specific – Sensor reacts with a single chemical, rather than a class of chemicals, for reliable detection without false positives.**
- **Sensitive – Ultra-low concentration detected.**
- **Flexible – DNA sensors can be developed for many different compounds.**
- **Convenient – An array of such sensors could provide fast detection of a number of different chemicals without cumbersome equipment.**



LTM - Biosensors

US Army Engineer Research & Development Center

Personnel: Shana Dalton

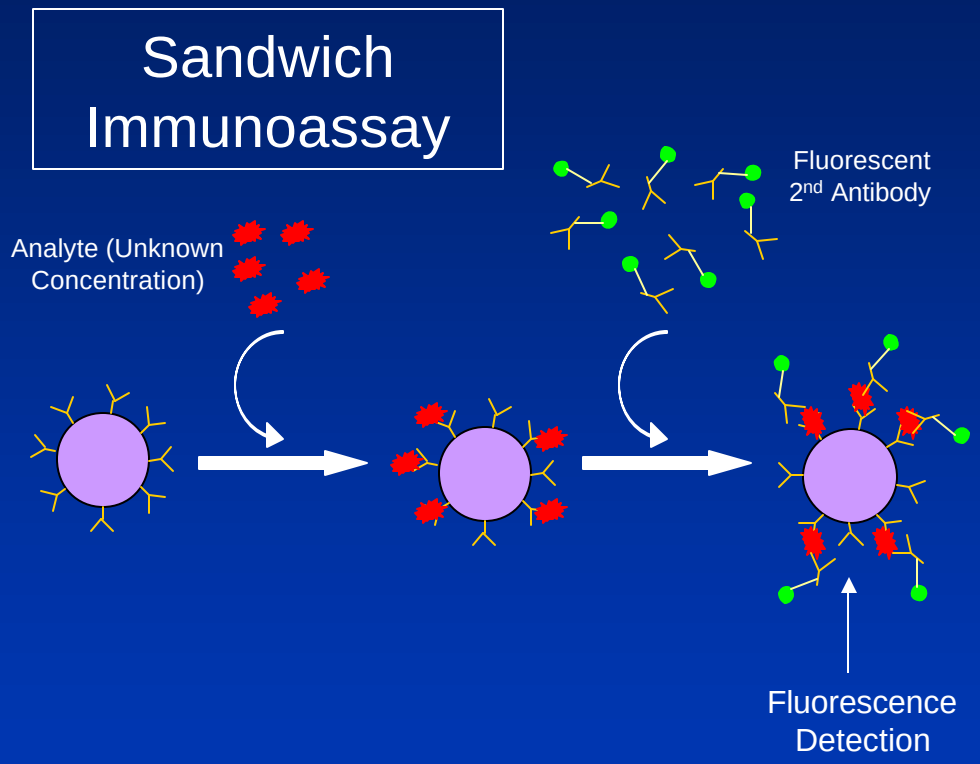
Objective - Develop field-portable and/or field-deployable biosensors for the detection of MUCs in groundwater.

- **Biological-based versus Analytical-based detection systems**
- **Whole organism biosensors (screening)**
- **DNA ?!**

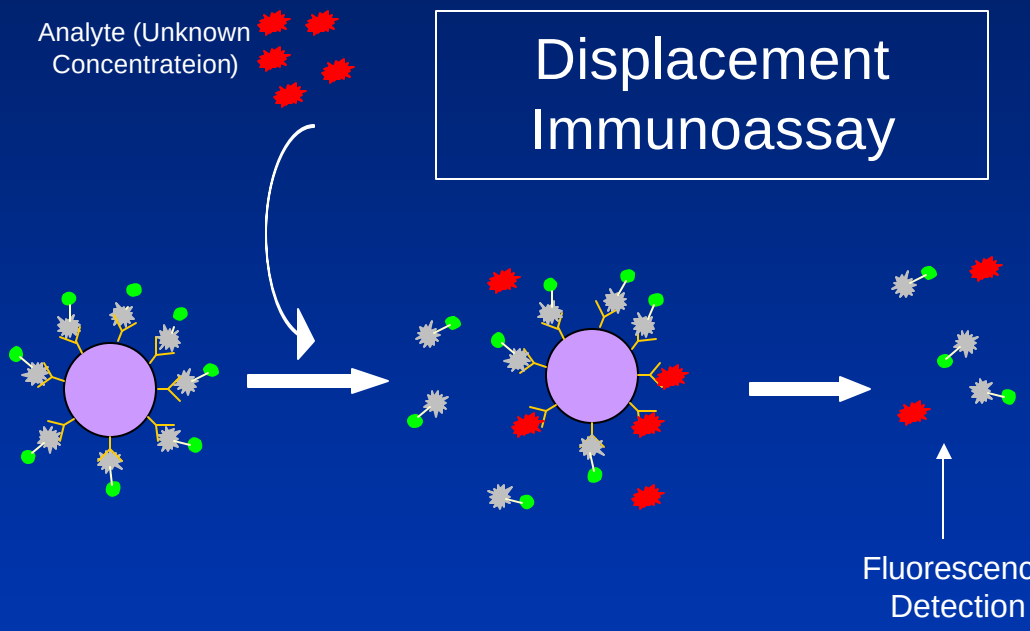
LTM - Biosensors

- Antibody-based technologies
  - Rapid
  - Sensitive
  - Specific
  - Small
  - Flexible / Adaptable
- Three primary techniques of immunoassays
  - Sandwich
  - Displacement
  - Competitive
- Fluorescent-linked detection

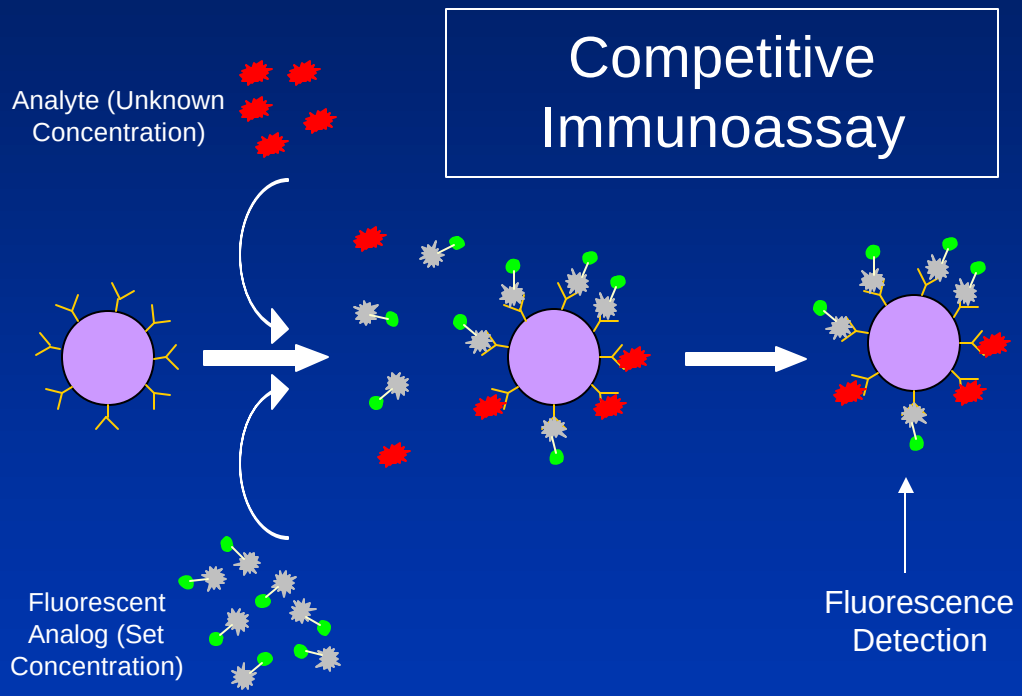
LTM - Biosensors



LTM - Biosensors



LTM - Biosensors



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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**
- **Collaborate with other laboratories currently developing immunoassay-based technologies**

LTM - Biosensors

- Magnetic bead experiments
  - RDX and TNT antibodies are commercially available (SBS)
  - Immobilize antibodies onto surface of beads
  - Produce fluorescently-tagged analogs
- Immunoassays with magnetic beads
  - Competition
  - Displacement
- Integrate beads into biosensors

LTM - Biosensors

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LTM - Biosensors

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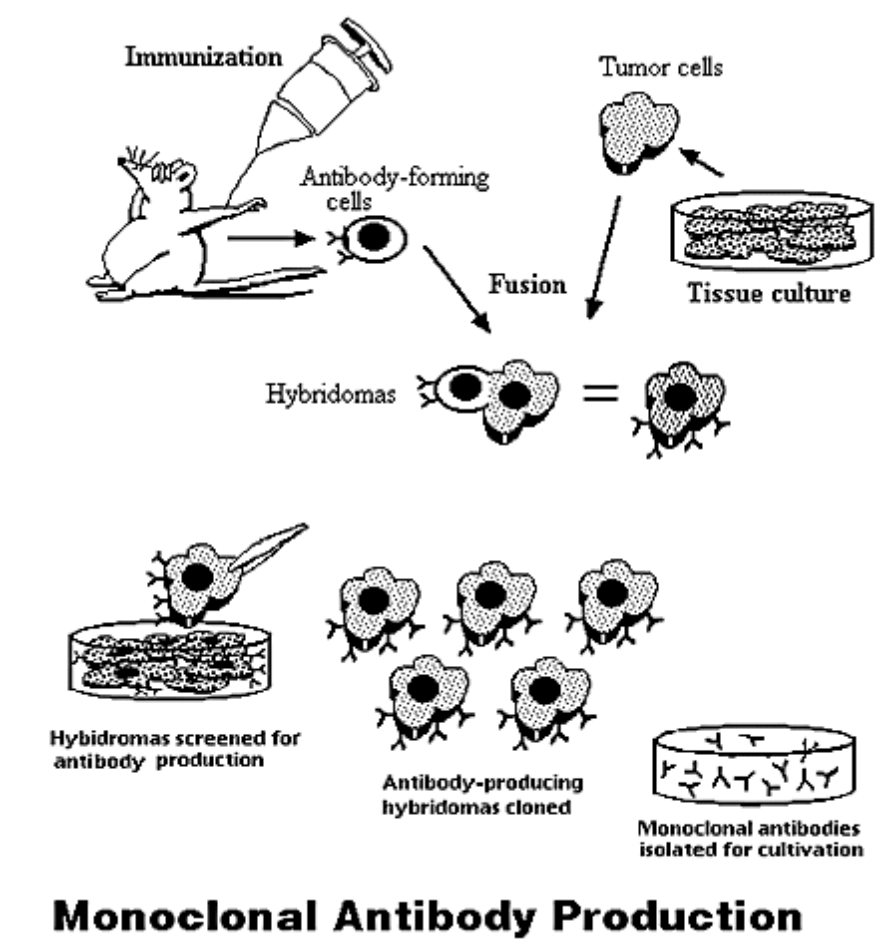
Hybridoma Development
(Strategic Biosolutions)

- Time Frame -
  5 to 9 months per analyte

- Costs -
  $7,500 - $15,000 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

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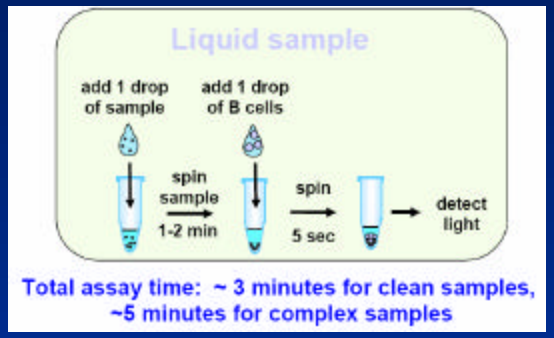
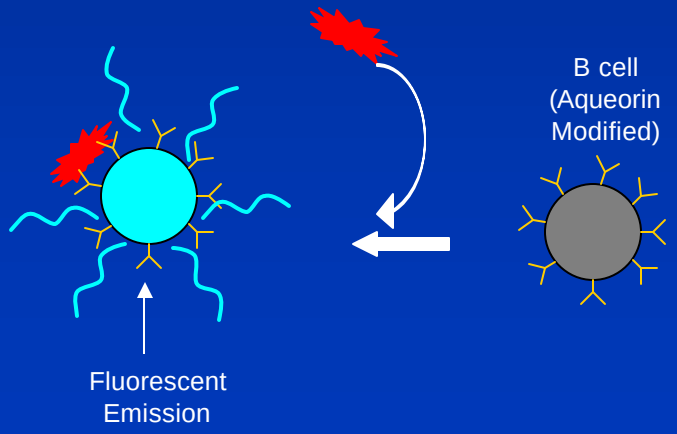
CANARY (Cellular Analysis and Notification of Antigen Risk and Yields)

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



Total assay time: ~ 3 minutes for clean samples, ~5 minutes for complex samples

CANARY Bioassay



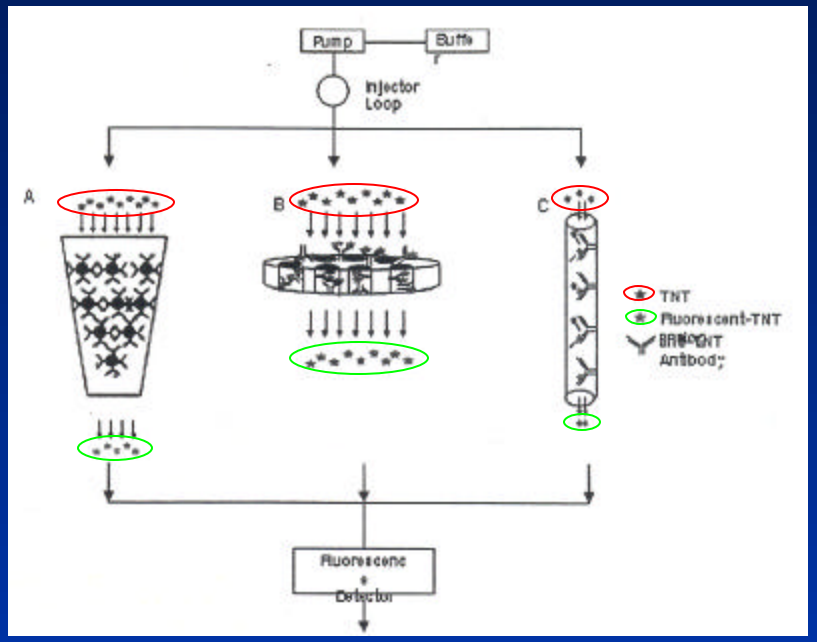
Source: Presentation at Federal Bio-Chem Detection Conference, Oct. 2003 by Peter Emanuel, PhD, Critical Reagents Program Director, JPE-CBD

LTM - Biosensors

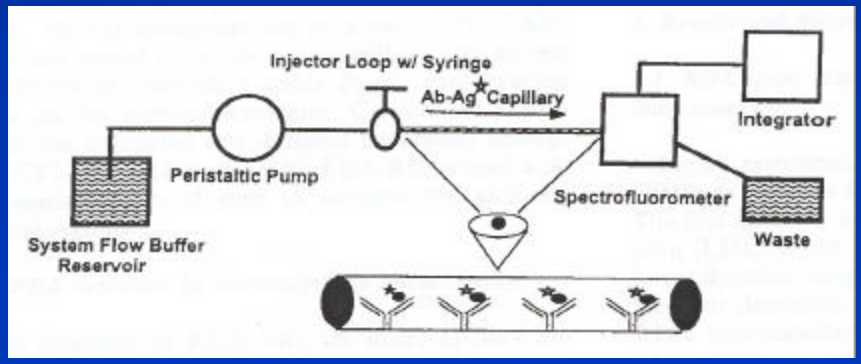
US Army Engineer Research & Development Center

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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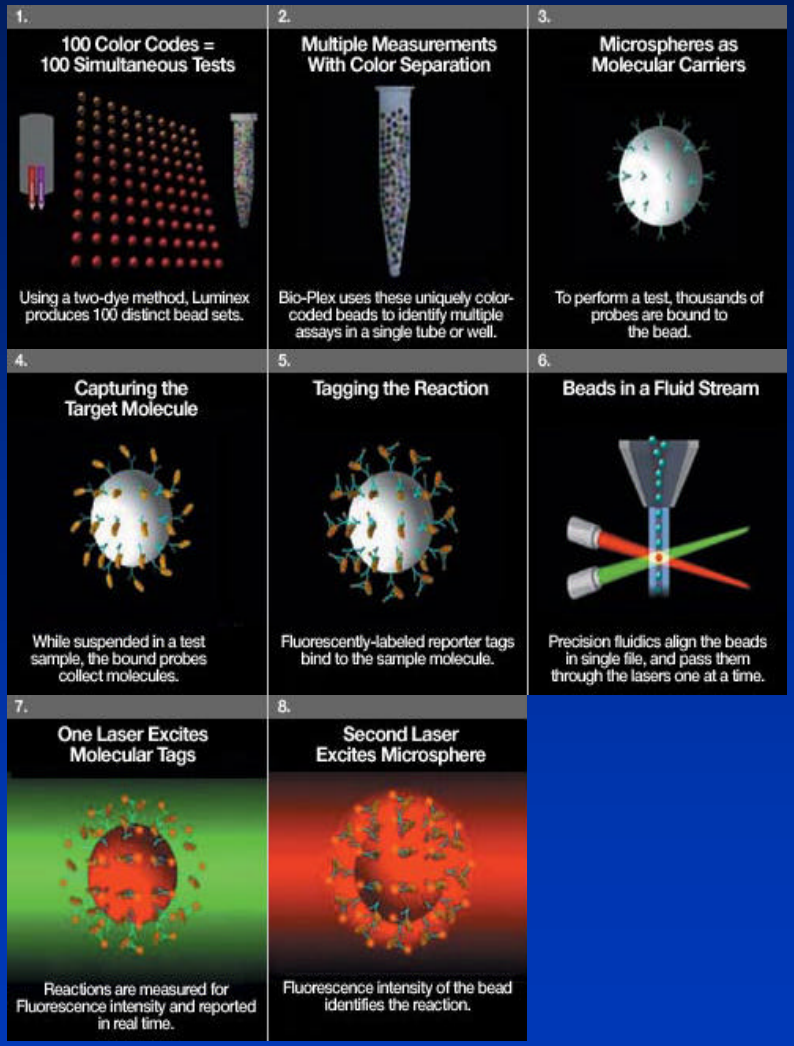
- Luminex® xMAP™ (Multi-Analyte Profiling) Technology
  - 100 unique analytes per assay
  - Minimal sample requirements



LTM - Biosensors

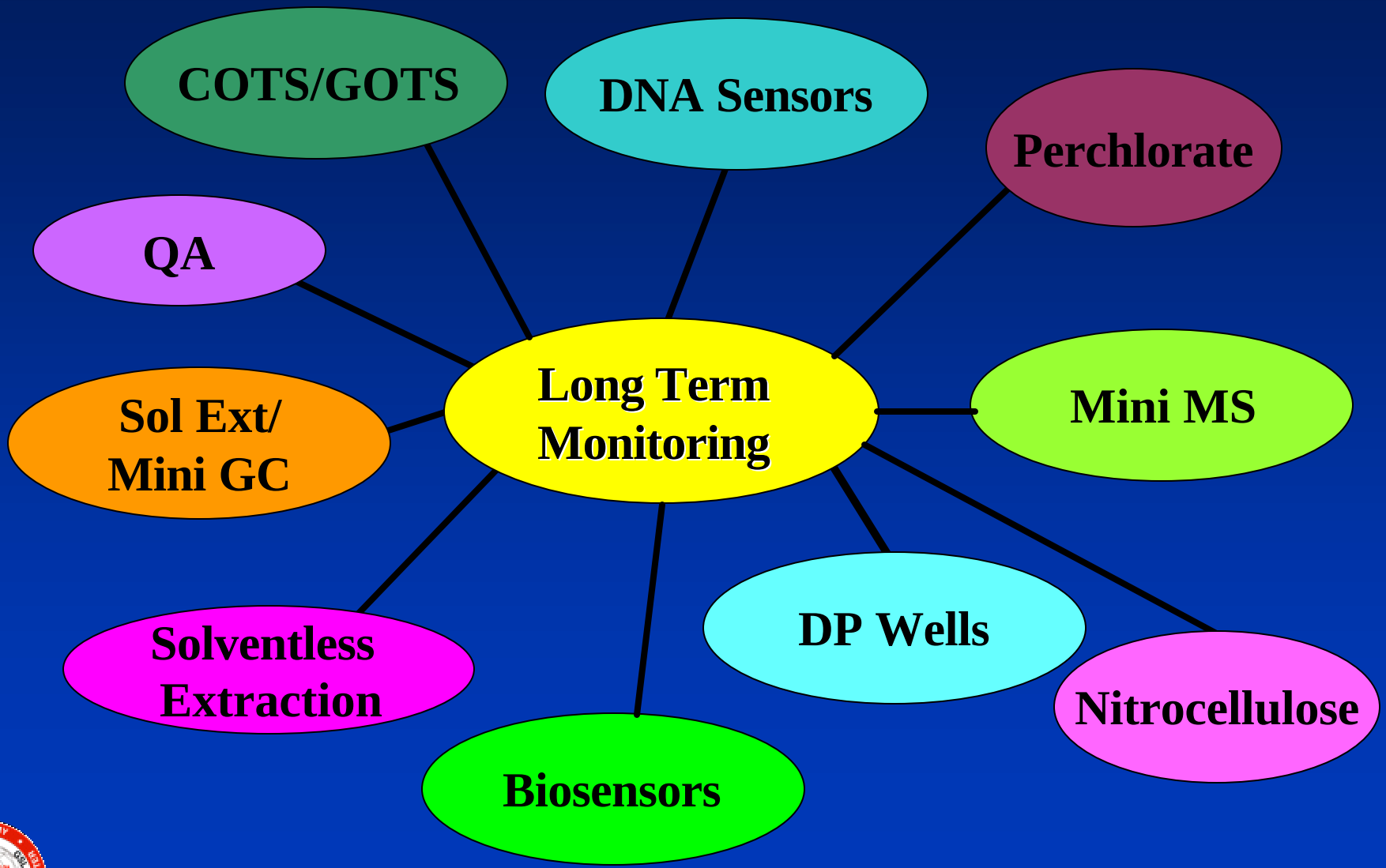
- Issues facing development of antibody-based biosensors…
  - Availability of Analogs for Small Molecules
  - Production of Antibodies to Small Molecules
  - Suitability of Method/Design for Detection of Small Molecules

LTM Project

The goals of the LTM project are being met by...

- **New protocols**
- **Modification of COTS/GOTS**
- **New and emerging technologies for a real-time field/site monitoring of groundwater for MUCs**

Long Term Monitoring Focus Area



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

LTM Project

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Acknowledgements

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