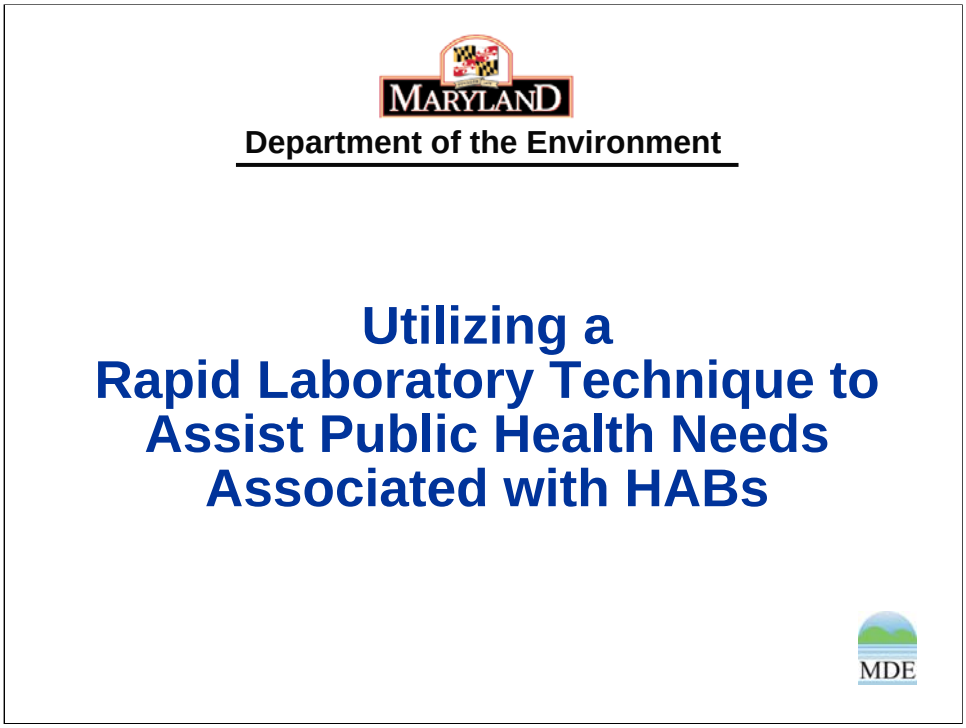


In 2009, a 3 yr cdc grant was awarded to the Sci Services Admin to purchase the equip to perform the MC ELISA test in the MDE wet lab,

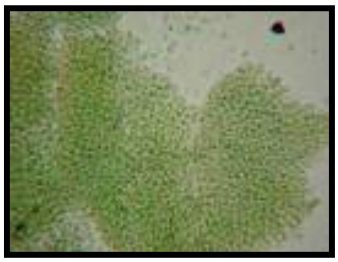
supporting the work of the inter-agency advisory group (MDE DNR DHMH)that manages the Harmful Algae Bloom surveillance program for Maryland.

Microcystis aeruginosa, can be harmful to humans and animals that come in contact with contaminated surface water,

Having this additional tool has significantly decreased delays in getting info needed to make timely mgt decisions to protect public health.

OVERVIEW

- **Microcystis cyanobacteria**
- **Microcystin toxin**
- **Enzyme Linked Immuno-Sorbent Assay**
- **Events**
- **Statistical Analysis**
  - **cell counts & ppb**



The entire presentation today revolves around MC aer - a freshwater blue green algae, capable of producing the hepatotoxin MC

Focus on an explanation of the ELISA Technology and few major HAB event from summer 2009 .

Finally, look at some statistics came out of the results, to see what can be used in future mgt decisions

ELISA Technology

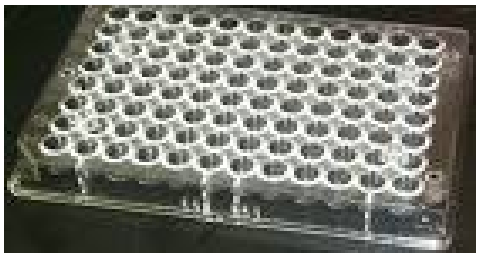
- **Abraxis, LLC – microtiter well system**
- **Delaware, Nebraska DEQ, USGS**

ELISA that we use comes as an all-inclusive test kit that is supplied by Abbraxis. And employes the micro-titer plate system

And is based on the identification of the toxin by an anitbody against MC.

ELISA Technology

- **Polyclonal AB – allows congener detection of MC and nodularins.**
- **Sensitivity Limit: 0.1 ppb**
- **Assay range: 0.15 – 5.0 ppb**



The AB is poly clonal – meaning it recognizes and binds with at least 8 differ congners of MC.

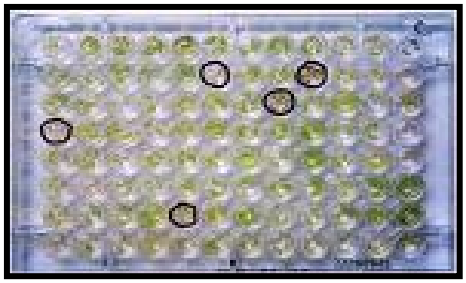
It can detect MC toxin in an env samples down to 0.1 ppb.

The reportable range for each run is based on conc of standards, from 0.150-05.0 ppb.

Sample dilutions were made, when necessary, to achieve that range.

Indirect competitive ELISA

- **Analogue (MC-LR)**
- **Environmental Sample**
- **+ AB + enzyme (HRP) + substrate (TMB) = color development**



In the MC ELISA microtiter plate sandwich immunoassay, the most common congener, MC LR, is adsorbed(BOUND) onto wells in a plastic microtiter plate. The treated test sample is added to the plate. When a polyclonal antibody is added in the next step, there is competition bet the bound toxin and the environmental sample toxin for binding sites on the antibody. So you can see, the lower toxin in the envir sample, the more antibody is avail to bind to the toxin coated onto the well. The converse is also true.
This binding reaction can then be measured by enzymes-substrate system. The more AB that remains in the well , the more color development.

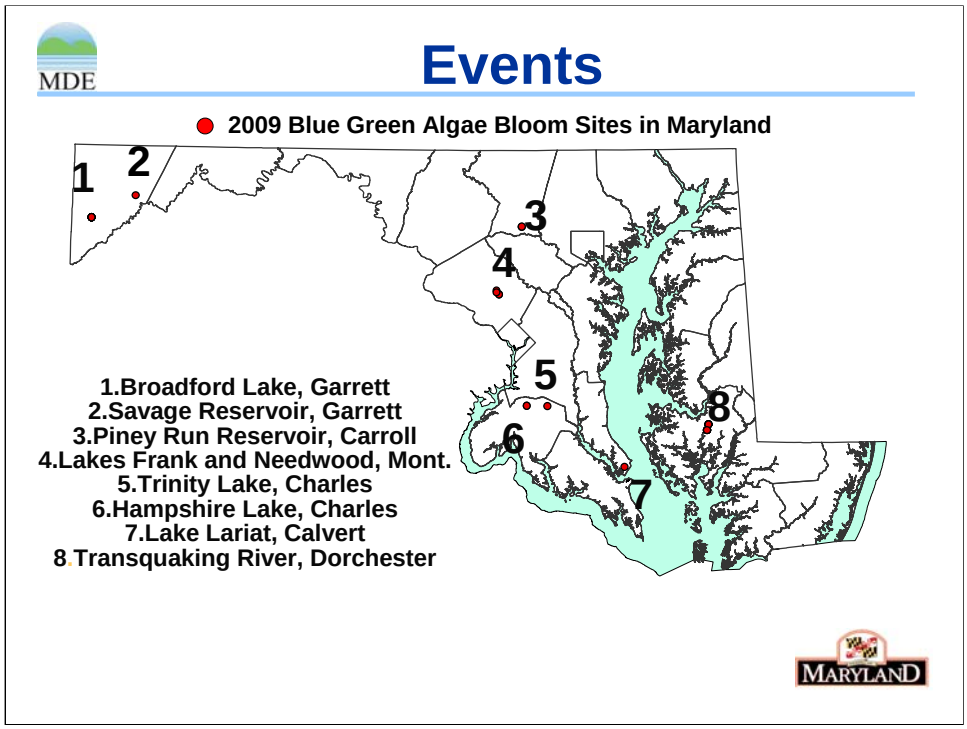
The color development system includes:

(TMB (3,3´,5,5´-tetramethylbenzidine) as a chromogen, hydrogen peroxide (and HRP). This produces a blue color. The color then changes to yellow with the addition of a stop soln. (sulfuric or phosphoric acid) and read on spect with maximum absorbance at 450 nm.[1,].

ELISA TECHNOLOGY

- **Read spectro-photometrically**
- **Compared to standard curve, concentration inverse to color intensity**

This system generates a color signal inversely proportional to the amount of target antigen present in the original sample added to the plate.



For over 30 yrs the state has been investigating HAB in the upper Bay, on the Potomac Ponds, and several ponds.

In 2009, MDE Investigated 29 HAB, 8 of those events involved MC aerug.

Events

- **2009, 8 individual events, 46 samples**
  - **May, Trinity Lake, Charles County**
  - **Summer/Fall, Transquaking River, Dorchester**
  - **Nov., Lake Needwood, Montgomery County**
- **15 greater than WHO guidelines for recreational waters (10 ppb) and 1 exceeded the drinking water standard (1.0 ppb)**

Events

Broadford Lake, Garrett County, July, 2009

Broadford Lake, a drinking water reservoir, also used for recreat..in W MD Garrett Ciounty

Events

- **Broadford Lake, Garrett County, July, 2009**
  - **Beach sample exceeded WHO guidelines for drinking and recreational waters**
  - **Initiative to coordinate with local Health Department and drinking-water plant personnel.**
  - **Advisory for water contact**
  - ***** Raw water analyzed for MC was below guidelines for drinking water – intake below surface**

In July, a green scum was observed at Swimming Beech area

Advisory and monitoring continued until toxin levels returned to safe drinking water levels.

Events

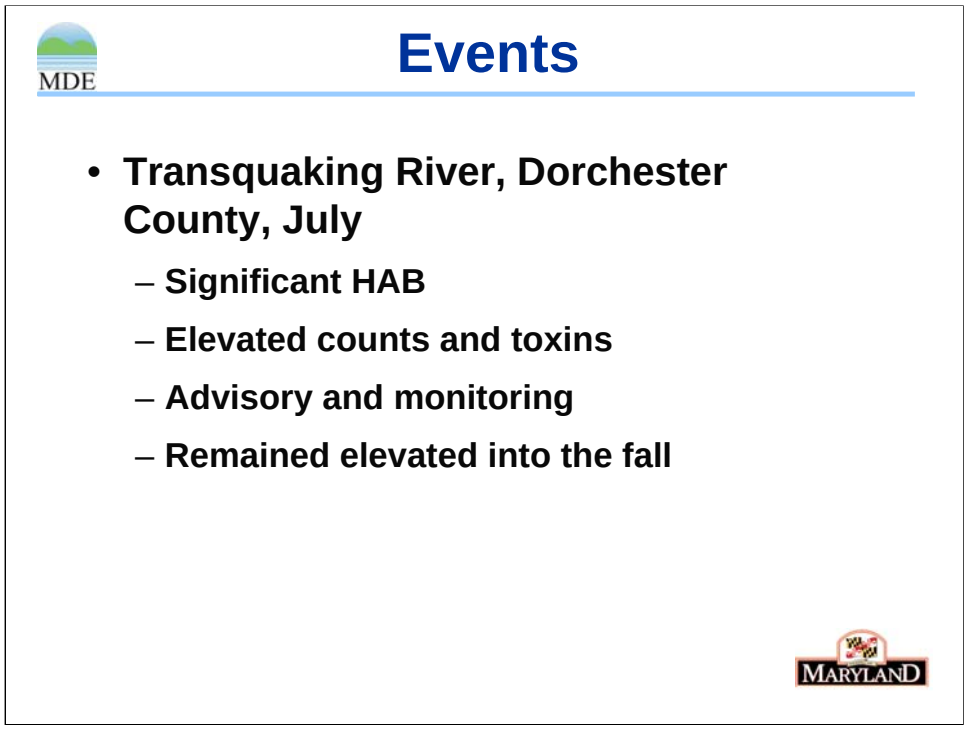
Transquaking River, Dorchester County

The next notable case occurred also in July, on HMP on the TRQ in Dorchest Cty.

Site of routine monitoring for algae, The Trq River has had HAB occur in the past and is routinely monitored a various sites,

near the dam, the drawbridge and in the pond.

picture shows a variety of land uses that could contrib to excess nutrients



when two dogs died unexpectedly after swimming in the Transquaking River, in Higgins Mill Pond,where a green scum occurred.

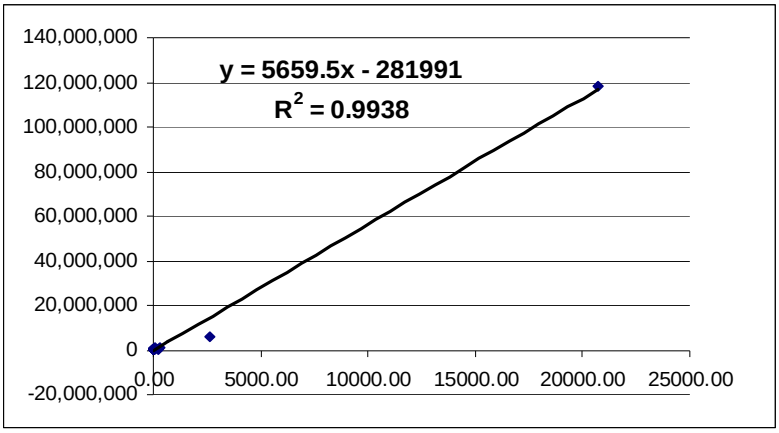
Again, Inter-agency cooperation at state and local levels, helped to reveal a MC bloom that contain elevated amounts of both algal cells and toxin.

Dorchester County health officials issued advisories and monitoring continued into the fall as elevated levels persisted. Toxin levels dissipated as the cooler weather set in.

Events

- March 2010
- Fountain Rock Quarry
- Plankothrix rubescens
- 5.6 mil cells/ml
- 500 ppb MC

Developing Bloom Threshold for Management Consideration

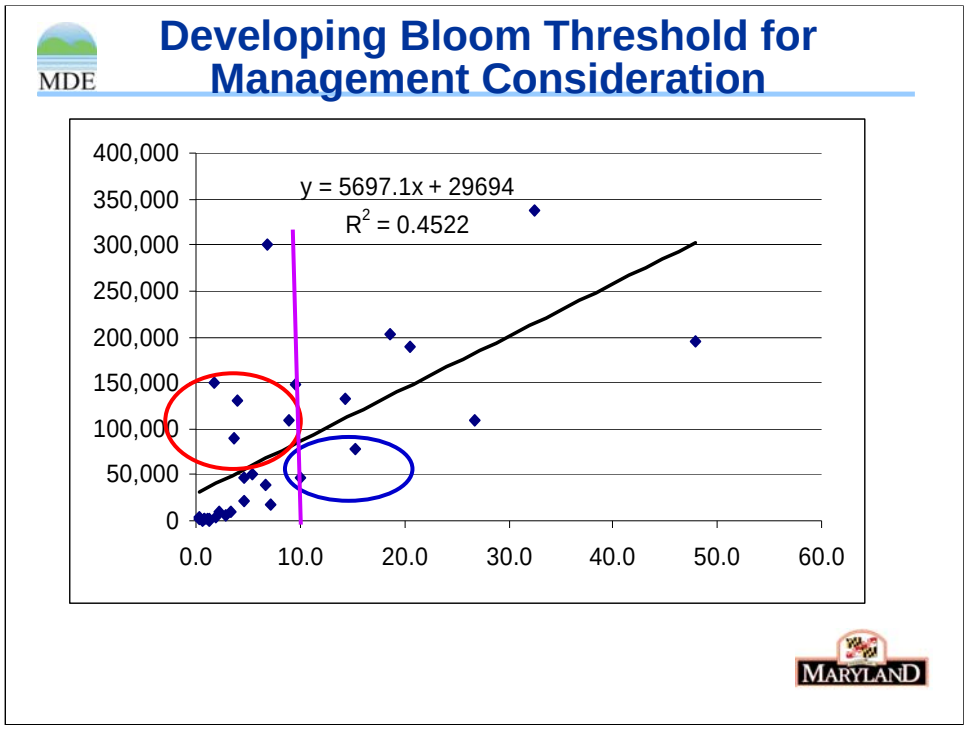


R sq values is high where we want it to be show good relation bet counts and ELISA values.

Look at the scale start at 20Mil,

Caused a cluster of 40 + points

Need to expand the cluster



R sq values is different,

Red cir – cell counts 90-150 k, ELISA LT 10.0 ppb

Blue cir – cell counts LT 100k, ELISA GT 10 ppb

One season of data pt, trends are developing,

Greater than 300k counts, ELISA GT 10 ppb, may want to issue advisory

Less than 300k, need extra testing.

Need to follow this over next two seasons, connecttion to other blue greens.

Developing Bloom Threshold for Management Consideration

- **2004, Dyble, found that:**
- **Microcystis cell densities only explained 34% of the variation in total microcystin concentration**
- **Several factors likely contribute to this poor correlation,**
- **(1) the inclusion of both toxic and nontoxic strains of Microcystis in cell counts,**
- **(2) variability in toxin production within a toxic strain related to cell growth, and**
- **(3) changing proportions of toxic to nontoxic genotypes in bloom populations**

- **Microcystin Concentrations and Genetic Diversity of Microcystis in the Lower Great Lakes**
- Julianne Dyble, et al, NOAA, Great Lakes Environmental Research Laboratory, 2205 Commonwealth Blvd, 2004

Quality control

Collection date 2009	Sample	SUNY PPIA(ug/L)	SUNY PPIA(ug/g)	MDE ELISA (ppb)
8/14	Higgins Mill pond		28918.9	20700
11/9	Savage Reservoir	< 0.200		0.25
11/19	Lake Frank	< 1.200		1.46
11/19	Lake Frank	< 2.857		2.94
11/19	Lake Needwood	37.616		48
11/19	Lake Needwood	< 12.000		4.6

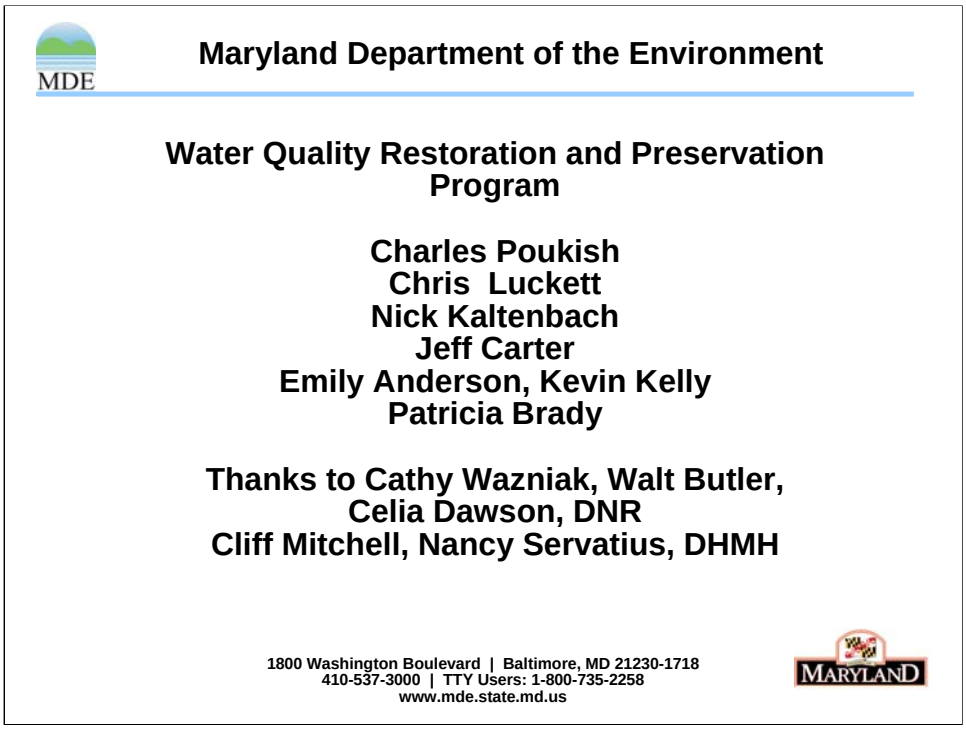
Inter-agency Cooperation

- ELISA decreased delays in management decision to protect public health

Results confirm fact that eutrophication can be a concern for public health.

ELISA is an effective and time-saving tool to manage the decision making process,

at state and local levels to protect public health from dangers associated with HABs.



Determine the best close for your audience and your presentation. Close with a summary; offer options; recommend a strategy; suggest a plan; set a goal. Keep your focus throughout your presentation, and you will more likely achieve your purpose.