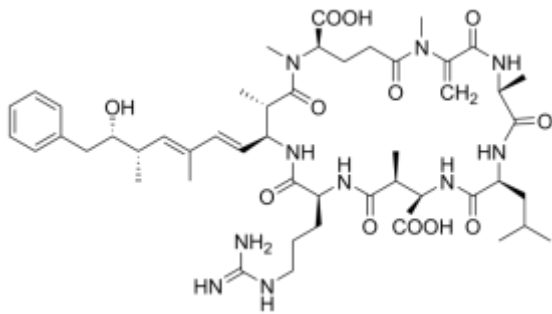
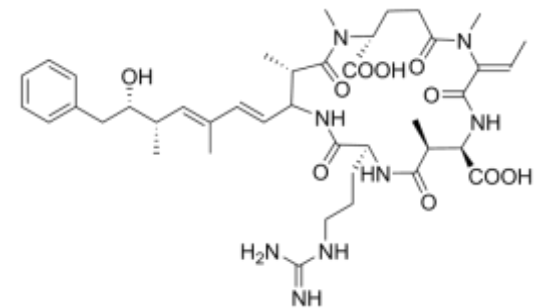


# Enzyme-Linked Immunosorbent Assay (ELISA)

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## Cyanotoxin Detection



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Spring 2009



# Biochemical Cyanotoxin Detection

- Replaced bioassays for rapid screening
- Less qualitative than physiochemical techniques but just as sensitive and more rapid (< 45 minutes)
- Very sensitive
  - LOD from 0.05 -0.5 ppb
- Less processing/extraction
- Variety of environmental matrices
- Commercially available kits for field & lab



# Anti-microcystin Antibodies

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- Based on polyclonal & monoclonal antibodies against Microcystin-LR (MCYST-LR)  
(Brooks & Codd 1988, Nataga et al. 1995, 1997)
- Cross reactivity against over 90 MCYST variants and nodularins (An & Carmichael 1994)
  - Not all variants & nodularins are equally responsive
  - Some nontoxic MCYST & nodularins have high affinity to antibody & more toxic MCYST less affinity
- Recommended to perform ELISA in conjunction with Protein Phosphatase Inhibition Assay PPIA for bioactivity/toxicity assay to help define actual risk/toxicity
- Results reported in MCYST-LR equivalents

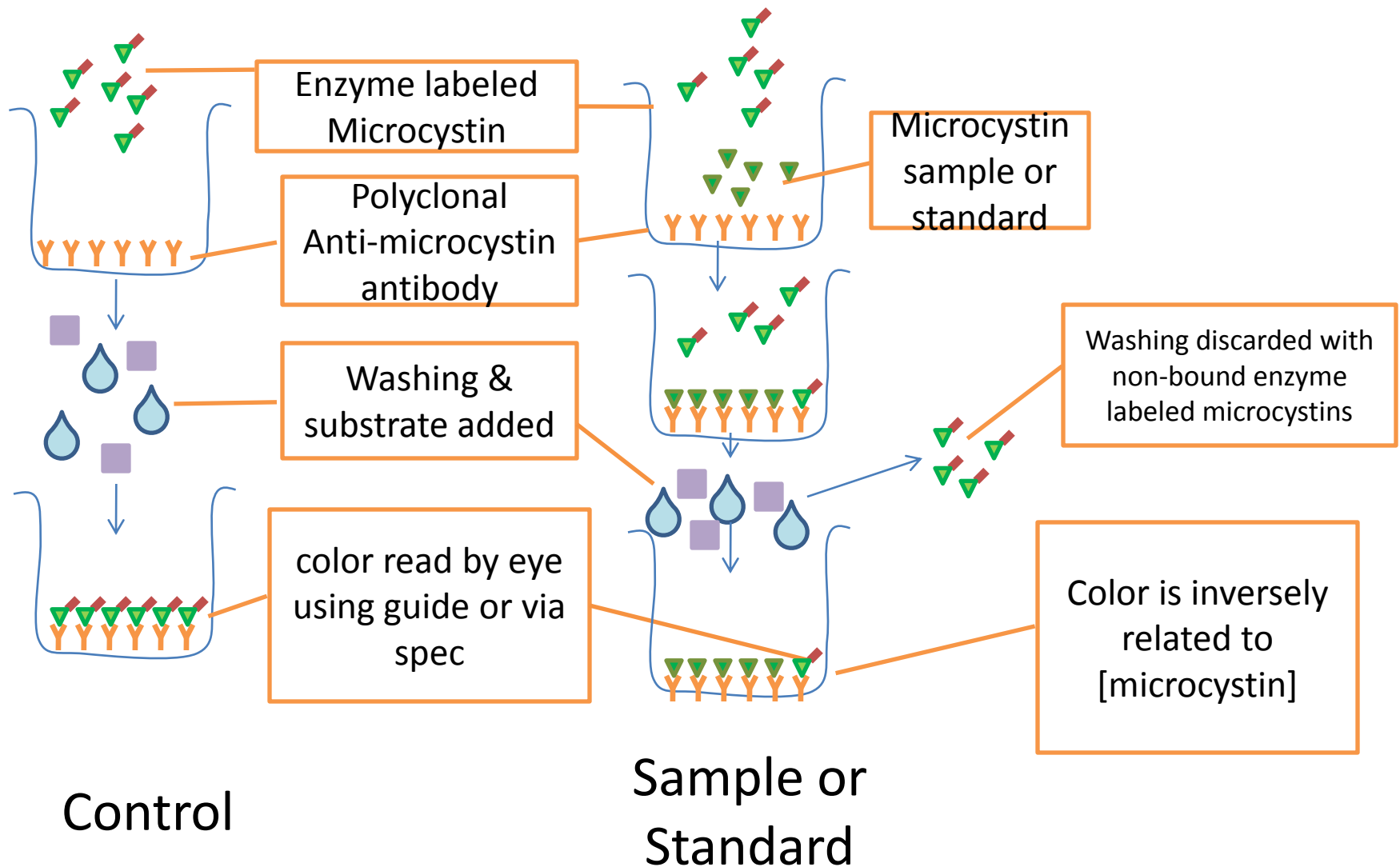
# Direct Competitive ELISA

## Polyclonal Antibodies

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- Polyclonal anti-microcystin antibodies on plate
- MCYST-LR used as a standard
  - Calibrated with blank & multiple concentrations
- MCYST-LR-peroxidase competes with MC-LR for binding sites of the antibody on the plate
- Incubation, washing & substrate added to develop color
- Color development = inverse to [MCYST]
- Read at 490 nm or estimated by color range

# Direct Polyclonal Competitive ELISA



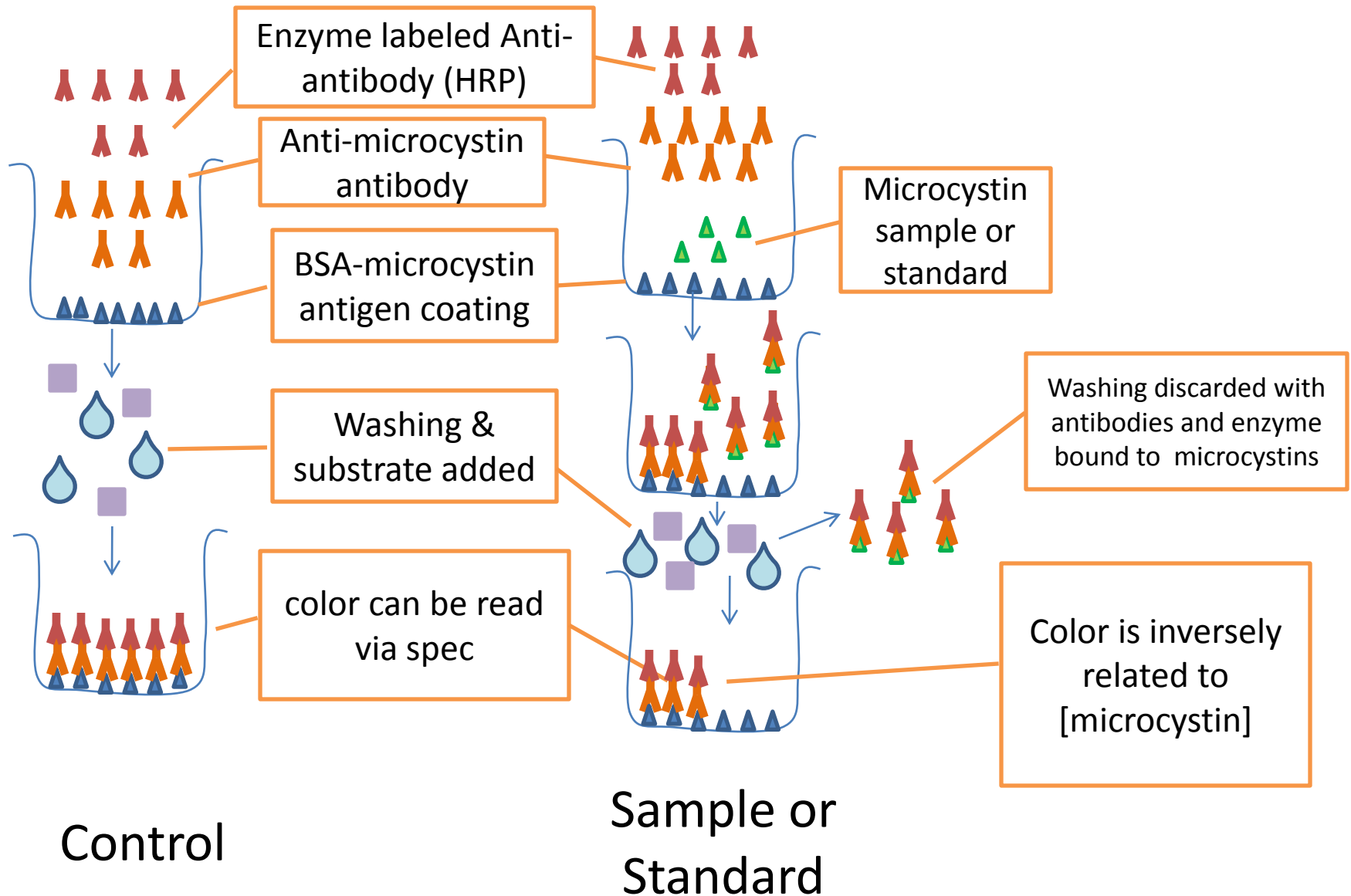
# Indirect Competitive ELISA

## Monoclonal Antibodies

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- Microcystin-LR-Bovine Serum Albumin (non specific antigen) on plate
- MCYST-LR is used as the standard
- 1<sup>st</sup> : add Monoclonal anti-MCYST-LR antibody competes with sample MCYST
- 2<sup>nd</sup> : Horseradish peroxidase (HRP) added to develop color
- Color development = inverse to [MCYST]

# Indirect Monoclonal Competitive ELISA



# Direct or Indirect?

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- Direct is currently less expensive
- Indirect has higher sensitivity, but needs more time and more processing
- Direct method can be performed in situ

# Environmental Samples

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- Water samples: separated by filtration and each fraction analyzed
  - Cell MCYST vs. water
- Animal tissue samples: organ tissues through procedure including extraction with 100% methanol, centrifuge, solid phase extraction and dilution

# Qualitube Kit :

Direct inhibition assay with polyclonal antibodies

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- Used by some Counties for regional lakes
- Field & lab kits
  - Field LOD 0.18 -0.3ppb, results in 40 minutes
  - Lab LOD 0.15 ppb, results in 2 hours
- Cross reactive for MCYST-LR, MCYST-LA, MCYST-RR, MCYST-YR, Nodularins
- Surface & drinking water samples

# Concerns with ELISA

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- Only a few MCYSTS & Nodularins available as standard compounds
- Analytic methods may overlook toxins & give false negatives
- Results given in equivalence units of standard
- Monoclonal ELISA with broad applicability?
- Time-resolved fluorescence immunoassay (TRFIA) and europium-labeled antimouse IgG conjugate (Lei et al. 2004)

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