EVALUATION OF LABORATORY METHODS FOR THE ANALYSIS OF MICROCYSTINS

by

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ABSTRACT

Evaluation of Laboratory Methods for the Analysis of Microcystins

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Finding low cost, rapid tests to monitor microcystins in water is paramount to protect environmental and public health worldwide. Bioassays like Protein Phosphatase Inhibition Assay (PPIA) and Enzyme-linked Immunosorbent Assay (ELISA) have many advantages over liquid chromatography–tandem mass spectrometry (LC-MS/MS). Analytical cost per sample was found to be $136 by ELISA and $365 by LC-MS/MS. An agreement of 76% was found between ELISA and LC-MS/MS results from 2010 to 2012 (n=854) on the basis of Method Detection Limits (MDLs). Among samples with LC-MS/MS results >1.5µg/L, ELISA missed 3 samples in 2010, 1 in 2011 and none in 2012.

Correlation between PP2A and ELISA was strong (R^2=0.8155, p=0.8054, n=27) in surface water samples but non-existent in drinking water (R^2=0.0366, p=0.0665, n=38). PP2A was found useful for monitoring non-coloured surface water but not for drinking water. A 2-tier test system is proposed: tier-1 ELISA and tier-2 PP2A for surface water samples.

ACKNOWLEDGMENT

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Last but not least, thanks to my dear and wonderful husband, Nasir Shad. This thesis would not be a reality without his continuous support, encouragement, patience and understanding.

This thesis is dedicated to my loving husband, Nasir Shad.

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ABBREVIATIONS

Adda	Amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid
AWQI	Adverse Water Quality Incident
bw	body weight
CE	Capillary Electrophoresis
CAWQ	Canadian Association on Water Quality
DWS	Drinking Water System
dc-ELISA	Direct Competitive Enzyme Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
FL	Fluorescence
FAB	Fast atomic Bombardment
GC	Gas Chromatograph
HPLC-MS	High Performance Liquid Chromatography-Mass Spectrometry
HPLC-PDA	High Performance Liquid Chromatography-Photo Diode Array
HRP	Horse Radish Peroxidase
IDS	Integrated Divisional System
LD_{50}	Dose lethal to 50% of population
LOAEL	Lowest-Observed-Adverse-Effect Level
LC-(ESI) MS/MS	Liquid Chromatography-(Electrospray Ionization) Tandem Mass Spectrometry
LOD	Limit of Detection
LMOH	Local Medical Officer of Health
MOE	Ministry of the Environment
MW	Molecular Weight
MAC	Maximum Acceptable Concentration
MAB	Monoclonal Antibody
MC	Microcystins
MDL	Method Detection Limit
MNR	Ministry of Natural Resources
MALDI-TOF	Matrix-Assisted Laser Adsorption Ionization-Time Of Flight
NOAEL	No-observed-adverse-effect level
nAChRs	Nicotinic Acetylcholine Receptors
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
O. Reg. 169/03	Ontario Regulation – Ontario Drinking Water Quality Standards
O. Reg. 248/03	Ontario Regulation – Drinking Water Testing Services
PCR	Polymerase Chain Reaction
PDA	Photodiode Array
ODWQS	Ontario Drinking Water Quality Standard
SAC	Spills Action Centre
ESSD	Sciences & Standards Division
SDWA	*Safe Drinking Water Act*
TDI	Tolerable Daily Intake
TLC	Thin Layer Chromatography
UF	Uncertainty Factor
UV	Ultra Violet

VFDF	Very Fast Death Factor
WHO	World Health Organization
WS	Surface Water
WD	Drinking Water

1 Introduction

Cyanobacteria, also known as blue-green algae, pose an emerging issue due to their potential impacts on drinking water and recreational waters. Climate change and high nutrient loading have favoured the ecological niche of cyanobacteria which have been blooming worldwide with alarming frequency and increasing bloom size.

Cyanobacteria produce a variety of toxins including the neurotoxins; anatoxin-a, saxitoxins, and the hepatotoxins which are microcystins and nodularin. Microcystins are the most commonly detected cyanotoxins of major health concern in Ontario's surface and drinking water. There are over 90 variants of microcystins (Welker *et al.*, 2004), many of them are toxic but most of them are not well studied. Microcystin-LR (MC-LR) is the only variant regulated under the Ontario *Safe Drinking Water Act, 2002* (*SDWA*) while the rest of the 90+ variants are ignored. The Ontario drinking water standard for microcystin-LR is set at1.5µg/L (1.5ppb).

Until 2010, the only test method licensed by the Ministry of the Environment (MOE) for analysing microcystins in drinking water was liquid chromatography (electrospray ionization) – tandem mass spectrometry [LC(ESI)-MS/MS]. By 2009, bioassay such as enzyme-linked immunosorbent assay (ELISA) was started at MOE as an analytical method for microcystins in surface water. In order to support the *SDWA*, on August, 2010 the regulation *O. Reg. 248/03* (MOE, 2010a) was amended to allow using ELISA to screen drinking water, particularly treated drinking water. Raw drinking water samples and surface water samples are still being analysed by LC-MS/MS at MOE because they do not fall under drinking water regulations. Consequently, the full benefit of ELISA has not yet been availed for surface and source water samples analysis.

ELISA cannot quantify individual variants in a water sample. Instead, it measures all variants that cross-react with the antibodies. Since ELISA is capable of measuring total microcystins inclusive of multiple variants, the Ontario Drinking-Water Quality Standards Regulation (*O. Reg. 169/03*) should be changed again from focusing solely on microcystin-LR to total microcystins (MOE, 2002). This change will also alleviate the regulatory reliance on the LC-MS/MS method.

There remains one big obstacle to change the regulatory focus from a single variant to multiple variants; LC-MS/MS results do not always correlate with ELISA results. This is because LC-MS/MS could only detect the 4 to 5 (-LR, -YR, -RR, -LA) variants for which pure chemical standards are commercially available while ELISA antibodies cross-reacts with numerous variants and can measures total microcystins. Consequently, ELISA often produces higher positive results compared to LC-MS/MS. Regulatory authorities need to be convinced that ELISA result is reliable even when it disagrees with LC-MS/MS result. This research provides evidence to support the premise. A convincing approach to address any disagreement between ELISA and LC-MS/MS would be to introduce a third analytical method as an adjudicator.

Protein phosphates inhibition assay (PPIA) measures the bioactivity or toxicity of microcystins but not the structural component like ELISA does. By definition, all toxic variants of microcystins are detectable by PPIA. However, PPIA has its own limitations. It is not specific to microcystins; other non-microcystins protein phosphatase inhibitors are detectable too. A logical algorithm is hereby proposed: screen water samples with PPIA as a tier-1 test followed by ELISA as a tier-2 test on only PPIA positive samples. This 2 tier test algorithm would confirm whether the toxicity present in the sample is due to microcystins. In this manner, the current *O. Reg. 169/03* (MOE, 2002) is supported.

ELISA and PPIA in combination can give a clearer picture in term of stoichiometry and toxicity. The combined assays can be conducted on multiple samples using the same equipment and in the same duration. ELISA and PPIA have numerous advantages over LC-MS/MS in terms of lower cost, higher throughput, faster turnaround time, and commercially available kits. More importantly, these assays empower laboratories which lack LC-MS/MS facility in the private sector or municipal level to participate in the surveillance of cyanobacterial threat to public and environmental health.

This thesis covers three aspects (1) scrutinize the agreement between ELISA and LC-MS/MS results (2010 to 2012) in the Ministry of the environment (MOE) data base (2) compare the costs between ELISA and LC-MS/MS at MOE (3) perform PPIA and ELISA, and determine the correlation between the two.

1.1 Hypothesis and Research Objectives

Microcystins are analysed at Ministry of the environment (MOE) by ELISA and LC-MS/MS. LC-MS/MS results do not always agree with ELISA results. LC-MS/MS could only detect the 4 to 5 variants for which pure chemical standards are commercially available. Many times water samples found positive by ELISA are negative by conventional LC-MS/MS. Hence, the following hypothesis is proposed:

Hypothesis: ELISA detects numerous variants of microcystins while LC-MS/MS detection is limited to few commercially available standards. Hence, ELISA produces more true positive results than LC-MS/MS.

The research objectives are as follows:

Objective 1: Analyse the discrepancy between ELISA and LC-MS/MS results from ministry of the environment (MOE) database over the period of three years (2010 to 2012).

Objective 2: Find a reliable, low cost and faster method (PPIA) for the analysis of microcystins to adjudicate the discrepancy between ELISA and LC-MS/MS results.

If this hypothesis proves to be correct and biochemical assays like ELISA and PPIA prove to be reliable methods for the analysis of microcystins, this thesis will provide the directions for more meaningful regulations and the logistic for sustainable monitoring program for drinking and surface water at the Ministry of the Environment (MOE).

2 Literature Review

2.1 Cyanobacteria and Cyanotoxins

Cyanobacteria are essential part of the ecosystem, and are natural inhabitants of aquatic systems. Their history is at least 3.5 billion years old (Altermann *et al.*, 2006) and they are responsible for consequential evolution of the Earth's lithosphere (Fischer, 1965; Altermann *et al.*, 2006).

Cyanobacteria have a simple structure at the subcellular level. They lack nucleus, a characteristics feature defining them, along with bacteria, as prokaryotes (Fogg *et al.*, 1973). The cyanobacteria also possess a photosynthetic apparatus enabling them to perform photosynthesis, but they lack chloroplasts, which differentiate them from algae and higher plants (Chorus & Bartman, 1999b). Their size is usually microscopic. However, when conditions are ideal, they reproduce rapidly and undergo a phenomenon known as "bloom" (clumps of cyanobacteria visible to the naked eye).

Blooms are symptoms of eutrophication and are evidence of the deterioration of water resources. They could be the result of effluent discharge, poor land and catchment management, and often of poor water allocation practices in rivers (Michael D Burch, 2009). Increased frequency and size of these blooms worldwide in fresh water are considered as an emerging threat to natural water reservoirs and drinking water supplies (Kaushik *at el.,* 2012).

Cyanobacteria produce a wide range of toxins which includes microcystins/nodularins, anatoxins, cylindrospermopsin, and saxitoxins (Frank, 2002), causing hepatotoxicity, neurotoxicity, cytotoxicity and dermatoxicity, respectively (Carmichael *et al.*, 1992). The details of these cyanotoxins with their health effects are summarized in the Table 1.

Microcystins has been found to be the most significant potential source of human injury world-wide. Therefore, the main study focus has been on microcystins. In southern Manitoba, microcystin-LR level of 0.1-0.6 µg/L was found at 44% of the sites (Jones *et al.*, 1998).

TABLE 1. GENERAL FEATURES OF THE CYANOTOXINS

Toxin group	Primary target organ in mammals	Cyanobacterial genera
Cyclic peptides		
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularin	Liver	*Nodularia*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Anatoxin-a (S)	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Aphanizomenon, Umezakia*
Lyngbyatoxin-a	Skin, gastro-intestinal tract	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides (LPS)	Potential irritant; affects any exposed tissue	All

Source: Chorus & Bartram, 1999a

Anatoxin-a, which is a neurotoxin, is another important cyanotoxins. They are not as common as microcystins. As a result, they are considered to be less of a concern for Canadian recreational waters (Agriculture Canada, 2012). However, they are always analysed along with microcystins in water samples submitted to the Ministry of the Environment (MOE). Anatoxin-a is considered more lethal than microcystins. It can cause death within minutes depending upon the amount of toxin an individual is exposed to (Wonnacott & Gallagher, 2006). There are no official guidelines available from Ontario or WHO (World Health Organization) or US EPA (Environmental Protection Agency) for this toxin.

Although human health impacts are of prime importance, another concern is the mortalities of aquatic and wild life caused by cyanobacteria. Mortality events, such as death of shellfish, fish, birds and mammals, have enormous impacts on local communities. For example, in 1987, a

phytoplankton bloom was linked to the mortality of 250,000 Atlantic salmon valued at over $500,000 (Ecohab, 2005).

Studies have also shown the negative impacts of cyanotoxins on plants. Microcystins can be taken up by plant seedlings; (Abe *et al.*, 1996) inhibiting the plant development, root growth and photosynthesis (Pflugmacher *et al.*, 2006). Necrotic lesions on the leaves were also observed due to microcystin-induced stress (Pflugmacher *et al.*, 2006).

On the other hand cyanobacteria have remarkable beneficial effects. They contribute significantly to global ecology by producing oxygen and fixing nitrogen. Cyanobacteria have chlorophyll a and photosystems I and II that allow them to perform oxygenic photosynthesis (Howells, 2008). Fossil records show that cyanobacteria played a primary role in the oxygenation of the Earth's atmosphere (Fischer, 1965; Altermann *et al*., 2006). However, increased N and P loading can contribute to an increased occurrence of cyanobacterial blooms in water. As the bloom subsides, the dead and decaying algae can reduce the oxygen levels in the water, causing stress or death of aquatic life.

Cyanobacteria are one of the main participants in nitrogen fixation in the ocean (Fong *et al.*, 2008). This ability of cyanobacteria to fix nitrogen has made it a very important agricultural asset. They are used as nitrogen fertilizer in the cultivation of rice and beans (Bold, 1985).

Cyanobacteria have gained a lot of attention because of their potential application for the treatment of HIV. An extract of *Arthrospiraplatensis* inhibits the HIV-1 replication in human (Ayehunie *et al.*, 1998).

The production of food supplements containing cyanobacteria is a growing industry worldwide (Saker *et al.*, 2007). Cyanobacteria are rich sources of proteins, lipids and vitamins (Howe, 1997).

Cyanobacteria are considered as good candidate for mass production of biofuel for the future (Skulberg *et al.*, 1995).

2.2 Environmental Factors Effecting Cyanobacteria

Cyanobacteria can form dense water blooms in the mesotrophic, eutrophic and hypertrophic water. The critical factors required for cyanobacterial growth are: stable water column, warm temperature, nitrogen, phosphorus, high pH and ample sunlight (Zuerwell, 2000; Haider *et al.*, 2003). Cyanobacteria blooms are more likely to occur in places where the water body is warm (Kong & Gao, 2005), shallow (Havens *et al.*, 1998), eutrophied (Vézie *et al.*, 2002), or slow moving.

Small vacuoles in the cyanobacteria allow them to regulate their buoyancy (Falcnor, 2005). Buoyancy enables them to migrate up and down the water column and to utilize nutrients confined to the sediments below (Falcnor, 2005). Cyanobacteria can multiply intensively in the form of blooms in still and stratified surface water (Falcnor, 2005). Buoyancy can be interfered by high water flows and high turbulence. When the wind stops, cyanobacteria may suddenly become over buoyant and form surface scum. Scum often forms overnight and may drift downwind towards the shores, where cyanobacteria may release toxins during decay (WHO, 1998). Hence, at the end of the warm season, cell lyses leads to increase toxicity in the water body.

2.3 Anatoxin-a

Anatoxin-a (Fig.1) is a low molecular weight neurotoxic alkaloid (MW = 165) produced by species belonging to the genera *Anabaena, Oscillatoria, Aphanizomenon* and *Cylindrospermopsins* species of cyanobacteria (Devlin *et al.*, 1977; Skulberg *et al.*, 1992; Chorus & Bartram, 1999; Falconer, 2005). This toxin is not very common but there are well recorded incidents of poisoning, usually fatal, of wild and domestic animals. Initially it was referred to VFDF (very fast death factor) and was associated with blooms of cyanobacteria in North America and in Europe (Wonnacott & Gallagher, 2006).

Anatoxin-a inhibits the cholinesterase with a mechanism similar to that of the organophosphorus insecticides (Van Apeldoorn *et al.*, 2007). It is a potent neuromuscular blocking agent that binds to neuronal nicotinic acetylcholine receptors at the neuromuscular junction. This blockage cause persistent stimulation resulting in secondary block of further electrical transmission across the synapse. These events can lead to muscular paralysis (Van Apeldoorn *et al.*, 2007). However, brain and retinal cholinesterase activities remain normal even in lethally poisoned animals (Briand *et al.*, 2003).

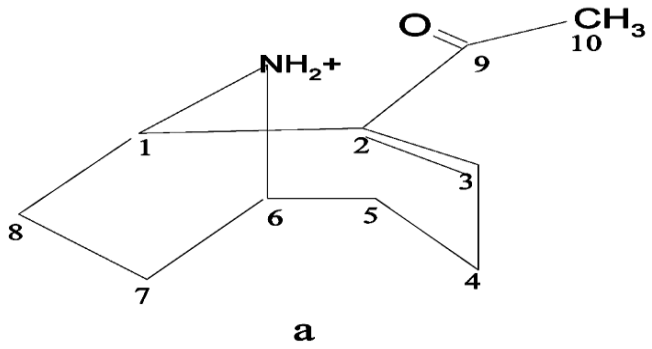

FIGURE 1. STRUCTURE OF ANATOXIN-A (CHORUS & BARTAM, 1999)

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In case of respiratory muscles, it can lead to death by asphyxiation within minutes to a few hours depending on the animal species and the amount of toxin exposed to (Carmichael, 2001; Van Apeldoorn, 2007; Falconer, 1999). Symptoms of anatoxin-a toxicity includes progression of muscle fasciculation, decreased movement, cyanosis, convulsions, cardiac arrhythmia and death (Carmichael, 2001; Fawell *et al.*, 1999).

The LD_{50} (lethal dose resulting in 50 per cent deaths) of anatoxin-a is 20 µg kg/ body weight (mouse) (Chorus & Bartram, 1999a; Carmichael *et al.*, 1990). The half-life for anatoxin-a breakdown was found to be approximately 14 days at pH 8 or pH 10, under normal day and night conditions (Smith & Sutton, 1993; Chorus & Bartram, 1999a).

2.3.1 TDI (Tolerable Daily Intake) of Anatoxin-a

NOAEL (No Observed Adverse Effects Level) of 98 µg/kg body weight per day of anatoxin-a was derived from a 28-day study in mice by Fawell *et al.*, (1999). Van *et al.*, (2007) calculated a

TDI of l.0µg/kg body weight by using an uncertainty factor of 1000 (100 for intra- and interspecies variation and 10 for limitations in the database).

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2.3.2 Regulations

No official guidelines are available from Ontario, WHO and US EPA. However, Fawell *et al.*, (1999) suggested a guideline limit of 1.0 µg/L of anatoxin-a for drinking water.

2.3.3 Detection Methods of Anatoxin-a

Methods for the detection of anatoxin-a are as follow:

2.3.3.1 Biological Assays

The mouse bioassay can be used to determine the minimum amount of toxin required to kill a mouse. When used to screen a sample, this method gives the total potential toxicity of the sample within few hours and makes it possible to distinguish hepatotoxins from neurotoxins (Carmichael, 1992). The lethal dose of the sample is compared to the lethal doses of known amount of toxins.

The disadvantages are: it is not sensitive enough to detect toxins at the microgram level (Harda *et al.*, 1996), and it does not identify the specific toxic agent (Lambert *et al.*, 1994a). This method had been obsolete due to lack of reliability and ethical implications (Fischer *et al.*, 2001).

2.3.3.2 Chromatographic Techniques

Anatoxin-a can be analysed by Gas Chromatography (GC) coupled to mass spectrometer (MS). Alternatively, it can be analysed by High Performance Liquid Chromatography (HPLC) using Ultra violet (UV) detector or Fluorescence (FL) detector or coupled to MS. Commercial standards are now readily available from Abraxis LLC for anatoxin-a (Abraxis, 2013a).

Although the Ontario *Safe Drinking Water Act 2002* (*SDWA, 2002*) does not stipulate any guideline for anatoxin-a, MOE proactively analyse for this toxin in routine water samples submitted for microcystins analysis. For this purpose LaSB use method # E3450 – Liquid Chromatography-(Electrospray Ionization) Tandem Mass Spectrometry [LC (ESI)-MS/MS] or LC-MS/MS in short. Anatoxin-a and microcystins are detected simultaneously by LC-MS/MS. Method detection limit (MDL) of anatoxin-a by LC-MS/MS is 0.02 µg/L (MOE, 2010a).

2.3.3.3 ELISA

Originally Anatoxin-a was detected in maize by ELISA with an LOD (Limit of Detection) of 1µg/kg (Young *et al.*, 2001). A receptor radioligand-binding assay was also used in the past to determine anatoxin-a in water with LOD close to 1.0 µg/L (Araoz *et al.*, 2005).

In April 2013, Abraxis (manufacturer) launched a commercial ELISA kit for the detection of anatoxin-a in water matrices. The detection limit is approximately 2.3ng/ml. The test is based on the affinity of anatoxin-a for nicotinic acetylcholine receptors (nAChRs) (Fig.2). Anatoxin-a competes with the biotinylated alpha-bungarotoxin for the acetylcholine binding sites of nAChRs which were coated on the microtiter plate. A streptavidin-horse reddish peroxidase (HRP) solution is added, if anatoxin-a is absent or low in concentration, the unbound nAChRs will be accessible to alpha-bungarotoxin (Abraxis, 2013a).

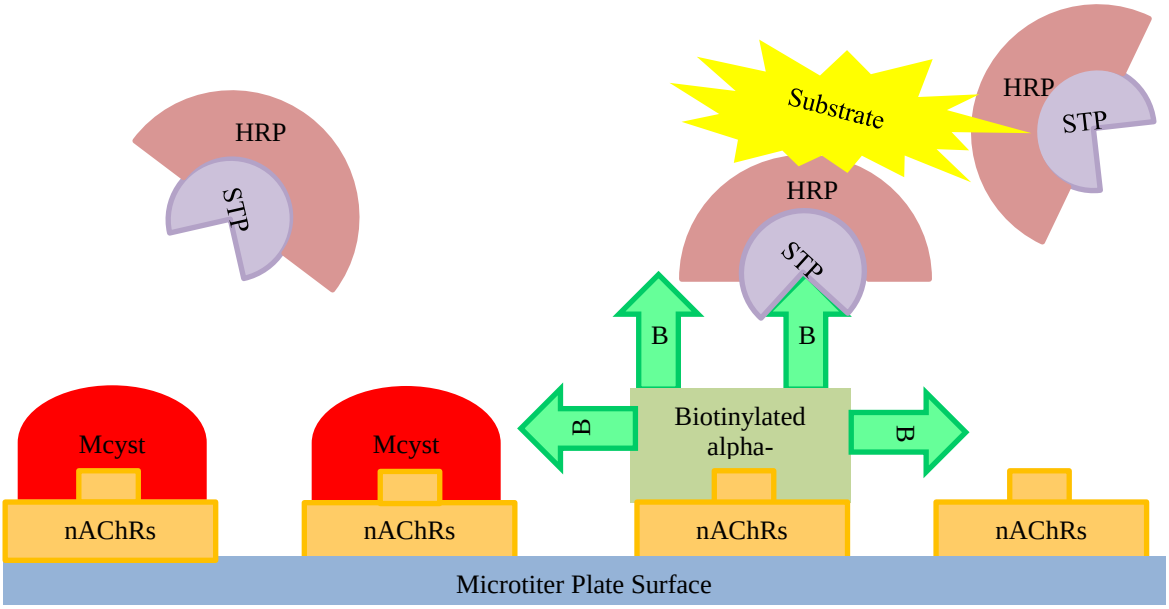

FIGURE 2. PRINCIPLE OF INDIRECT COMPETITIVE ELISA FOR THE DETECTION OF ANATOXIN-A

nAChRs=nicotinic acetylcholine receptors, B=Biotin, STP=Streptavidin, HRP=Horse Radish Peroxidase

The amount of biotinylated alpha-bungarotoxin bound to the remaining nAChRs is tagged by addition of streptavidin-HRP. Addition of chromogenic substrate produces a color reaction. The color intensity is measured by a spectrophotometer at 405nm. Since this a competitive reaction, the intensity of the blue color is inversely proportional to the concentration of anatoxin-a present

in the sample. The concentration of anatoxin-a is calculated by interpolating the absorbance of the sample on a standard curve (Abraxis, 2013a). Detection of anatoxin-a is by this simple and economical method performed in microtiter plate could potentially reduce the LC-MS/MS workload.

2.4 Microcystins

Microcystins are the most commonly found cyanobacterial toxins in the drinking and surface water. It is produced by cyanobacterial species belonging to the genera *Microcystis, Anabaena, Anabaenopsis, Oscillatgoria, Hapalosiphon* and *Nostoc* species of cyanobacteria (Chorus & Bartram, 1999a; Janse *et al.*, 2005). It is associated with hepatotoxicity (Chorus & Bartram, 1999a) and is a known tumor premotor (Falconer *et al.*, 1991).

2.4.1 Structure and Properties of Microcystins

Microcystins comprises a group of cyclic heptapeptide characterized by a unique amino acid ADDA (3-amino-9-methoxy-2, 6, 8- trimethyl-10-phenyldeca-4, 6-dienoic acid) which is present in > 80% of known toxin variants (Fischer *et al.*, 2001) (Fig.3). Variation of amino acids (Fig.3) at positions 2 and 4 (X and Z) provides the basis for microcystins nomenclature (Campos *et al.*, 2010). The range of molecular weight of microcystins is from 800 to 1100 Daltons (Gurbuz *et al.*, 2009). Eighty structural variants of microcystins have been characterized out of 90+ variants (Welker *et al.*, 2004). Some researchers used the term congeners to refer to the various chemical structures of microcystins. The term variant should be used because these are products of biological variations. The term congener should be reserved for chemical variations.

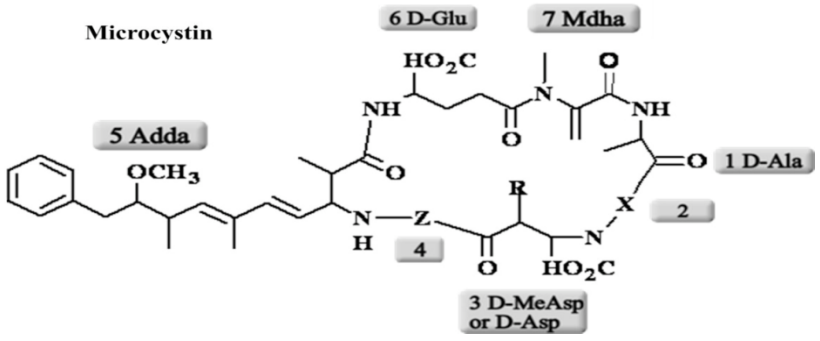

FIGURE 3. STRUCTURE OF MICROCYSTINS, WITH X AND Z REPRESENTING TWO AMINO ACID VARIABLES (SILVA-STENICO, 2009)

Microcystins is a potent inhibitors of the serine/threonine protein phosphatases type1 (PP1) and type 2 (PP2) enzymes (Honkanen *et al.*, 1990; MacKintosh *et al.*, 1990).

Among the numerous variant of microcystins, microcystin-LR was the first to be chemically identified. Most of the research work is conducted using this variant and most of the worldwide incidents are associated with its frequent occurrence. However, the -LA, -RR and -YR variants of microcystins have similar toxicological effects (EPA, 2009b). The molecular weight and positions of amino acids in the variants are given in Table 2.

TABLE 2. MICROCYSTINS VARIANTS WITH THEIR AMINO ACID IN POSITION X AND Y, AND MOLECULAR WEIGHT

Name	X-position Amino Acid	Z-position Amino Acid	Molecular Weight
Microcystin LA	Leucine (L)	Alanine (A)	910.06
Microcystin YR	Tyrosine (Y)	Arginine (R)	1045.19
Microcystin RR	Arginine (R)	Arginine (R)	1038.2
Microcystin LR	Leucine (L)	Arginine (R)	995.17

Source: EPA (2009b)

2.4.2 Health Effects

Microcystins are primarily a hepatotoxic (Chorus & Bartman, 1999a). It inhibits protein phosphatases type 1 (PP1) and type 2A (PP2A) enzymes (Falconer, 1991; Falconer *et al.*, 1992; Sekijima *et al.*, 1999; Mackintosh *et al.*, 1990). The metabolism of a cell relies on the function of numerous enzymes and proteins. These enzymes/proteins are normally in a resting state. Usually, phosphorylation is required to convert the enzyme/proteins from its resting state to its active state. Phosphorylation of protein and enzymes are achieved by protein kinases at the expense of adenosine triphosphate. After the enzyme has performed its necessary functions, the phosphate radical is removed by protein phosphatase type 1 or 2 in order to return the active enzyme into its original resting state (Fig.4). If the phosphate radical is not removed, the enzyme will remain active and the cell will enter a hyperactive state. The toxicity of microcystins is due to its covalent binding to phosphatases thereby inhibiting the dephosphorylation reaction. Consequently, microcystins can cause hyper phosphorylation of proteins, irreversible re-organization of cellular microfilaments and destruction of the liver cells (Rapala *et a.l,* 2002)

which can lead to blood accumulation in the liver and eventually can be fatal for the animal (Falconer *et al.*, 1999).

Acute exposure to cyanotoxins may cause mild symptoms; headache, vomiting, stomach cramp and skin rash (Chorus & Bartman, 1999c). Exposure could be from many sources such as drinking water, surface water, contaminated food or food supplements (Dittmann *et al.*, 2006). In Brazil, 52 patients died after hemodialysis from microcystins contaminated water (Jochimsen *et al.*, 1998).

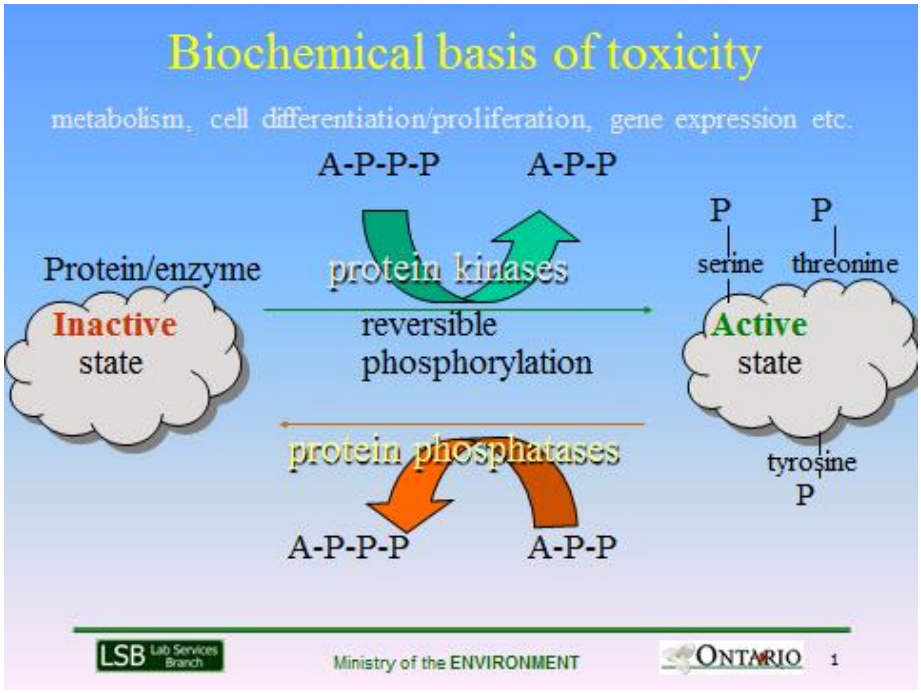

FIGURE 4. INTRACELLULAR PHOSPHORYLATION REACTION (REPRODUCED WITH PERMISSION FROM DR. CHING LO, MOE

Chronic exposures of microcystins have affected birds, amphibians, fish wild and domestic mammals from mild to fatal outcomes (Codd *et al.*, 2005). Consumption of water containing more than 10^6/mL of cyanobacterial cells had caused animal deaths (Carmichael, 1992, 1997). Microcystins can also enter the aquatic food chain by accumulating in the tissues (Lance *et al.*, 2007; Smith *et al.*, 2008). Therefore microcystins, which may not be toxic for fish, can accumulate in fish to the level that may lead to microcystins toxicity if consumed by human beings (Lindner *et al.*, 2004).

2.4.3 Stability of Microcystins

Microcystins is found in the bacterial cells in bound and free forms (Carmichael, 1992). The death of cyanobacteria releases both forms of the toxin into the water (Health Canada, 2013). It is water soluble. Microcystins is very persistent in the environment (Ressom, 1994) and difficult to degrade or remove. Its cyclic structure renders its chemical stability (Campas *et al.*, 2007). It can persist up to 2 to 3 months in water and 5 to 6 months in dried form (Ressom *et al.*, 1994). It degrades slowly in water at temperature higher than 40ºC at pH <1 or >9 (Harada *et al.*, 1996). Microcystin-LR and nodularin can reach a half-life of 4 to 18 days in water (Edwards *et al.*, 2008).

The removal of microcystins in nature is due to the following known processes. The bacterial Genus *Sphingomonas* have the capability of degrading microcystins (*Harda et al.*, 2004). Microcystins present in the cooler and dark areas of surface water can persist from months to years in the absence of these bacteria (Rapala *et al.*, 2005, Jones *et al.*, 1995). Another bacterium, *Spirodela intermedia* have also shown the ability to uptake microcystin-RR, confirmed by high pressure liquid chromatography – photodiode array (HPLC-PDA) analysis (Ferreira *et al.*, 2009). Pflugmacher *et al.*, (2001) also found the uptake of microcystin-LR by reed plants.

Microcystins cannot be destroyed by boiling (Health Canada, 2013). Ontario, Ministry of Environment recommends the best solution is prevention or discontinue the use of the contaminated water until the bloom is abated.

2.5 Guidelines for Microcystin-LR

The guidelines for the microcystin-LR in drinking water were introduced in 1998 by WHO and in 2002 by Ontario Ministry of the Environment and had not been changed since. At that time, the only sufficient information available was for microcystin-LR only. Hence, guidelines are based only on one variant (microcystin-LR) out of 90 (Welker *et al.*, 2004). The details of the Ontario and WHO drinking water guidelines are given below:

2.5.1 Ontario Drinking Water Guidelines

Maximum acceptable concentration (MAC) of microcystin-LR under *Ontario, Safe Drinking Water Act (SDWA),* 2002 - *O. Reg. 169/03* in drinking water is 1.5µg/L (MOE, 2002). This MAC is based on the tolerable daily intake (TDI) of microcystin-LR. The calculation of TDI and MAC is as follow:

a. TDI (Tolerable Daily Intake)

Microcystins has been placed in Group IIIB (Inadequate data in humans, limited evidence in experimental animals). Hence, the TDI calculations are based on LOAEL (Lowest Observed Adverse Effect Level) or NOAEL (No Observed Adverse Effect Level) from chronic and sub chronic studies, divided by an uncertainty factor to derive tolerable daily intake (TDI) (Health Canada, 2008).

The TDI derived for microcystin-LR is as follow:

$$TDI = \frac{NOAEL}{UF}$$

$$TDI = \frac{\frac{40\ \mu g}{Kg}\, bw\ per\ day}{1000} \quad = \frac{0.04 \mu g}{kg}\, bw\ per\ day$$

where:

NOAEL = No Observed Adverse Effects Level of 40 µg/kg bw (body weight) per day is derived from 13 week mouse study with liver changes at the Water Research Centre, the United Kingdom (Fawell *et al.*, 1994).

UF = 1000 is the uncertainty factor (×10 for intraspecies variation, ×10 for interspecies variation and ×10 for the less-than-lifetime study).

b. MAC (Maximum Acceptable Concentration) for microcystin-LR

The maximum acceptable concentration of 1.5µg/L is calculated from TDI is as follow:

$$MAC = TDI \times bw \times P/L$$

$$MAC = \frac{\frac{0.04\mu g}{Kg} bw\ per\ day \times 70Kgbw \times 0.80}{1.5L/D} = 1.5\mu g/L$$

TDI = 0.04 µg/kg bw per day, as derived above

bw = 70 kg bw is the average body weight of an adult

P = 0.80 is the proportion of total intake considered to be ingested in drinking water

L = 1.5 L/d is the average daily consumption of drinking water for an adult

The MAC of 1.5µg/L for microcystins is derived on the basis of daily consumption of microcystin-LR over a full year of a study. This calculated MAC for microcystin-LR is believed to be protective against exposure to the other microcystins variants too (Health Canada, 2008).

2.5.2 World Health Organization (WHO) Provisional Drinking Water Guidelines

Maximum Acceptable Concentration (MAC) of microcystin-LR in drinking water is 1.0 µg/L. This guideline value is provisional, as it covers only one variant i.e. microcystin-LR.

The TDI of 0.04 micrograms per kilogram body weight per day (µg/kg/d) calculated by WHO is also based on the same 13-week study of mice by Fawell *et al.*, (1994) which were used by Health Ontario, Canada to calculate the TDI. Hence, the calculated TDI is the same by both. However, MAC calculation varies (WHO, 1998).

The maximum acceptable concentration (MAC) was calculated as:

$$MAC = TDI \times bw \times P/L$$

$$MAC = \frac{\frac{0.04\mu g}{Kg} bw\ per\ day \times 60Kgbw \times 0.80}{2.0L/D} = 1.0\mu g/L$$

TDI = the Tolerable daily intake of 0.04 µg/kg bw per day, as derived by health Canada

bw = the average body weight of an adults; 60 kg (132lb)

P = the proportion of total intake considered to be ingested in drinking water used 0.80 or 80%

L = the average daily consumption of drinking water for an adult is 2.0 L/d

Guideline value was supported by a 44-days study on pigs, exposed to an extract from *M. aeruginosa* containing microcystin-LR (WHO, 1998). The risk level and guideline of microcystins by WHO is summarised in the Table 3.

TABLE 3. WORLD HEALTH ORGANIZATION (WHO) GUIDELINES AND RISK LEVELS FOR MICROCYSTINS

	Microcystins concentration	**Cyanobacteria cells/ml**
Tolerable Daily Intake (provisional)	0.04 µg/kg-day	
Recreational Water		
Low risk	4 µg/L	20,000
Moderate risk	20 µg/L	100,000
High risk		Scums
Drinking Water (provisional**)**	1 µg/L	

Source: Joan Hardy, (2008)

2.5.3 Drinking Water Guidelines from Other Countries

Most of the countries (e.g. Czech Republic, France, Japan, Korea, New Zealand, Norway, Poland, Brazil and Spain, USA) have adopted directly the WHO provisional guidelines. Some countries have adopted the same animal studies as by the WHO and modified it based upon their local requirements. For example, Canada has MAC of 1.5µg/L for microcystin-LR in the drinking water and Australia has MAC for total microcystins of 1.3µg/L as toxicity equivalents of microcystin-LR. Brazil set the most comprehensive mandatory standards that include the MAC of 1.0µg/L for microcystins, 3.0µg/L for saxitoxins (equivalents) and 15µg/L for cylindrospermopsin (Michael D Burch, 2009).

2.6 Cyanobacteria an Emerging Contaminant of Concern

Canada has almost two million lakes (biodivcanada, 2011) of 891,163 sq km area (Env Canada, 2011) which are third most renewable freshwater resources of the world (Env Canada, 2010). In 40th national conference of “Canadian Association on Water Quality” (CAWQ) held on Feb

12th, 2012, cyanotoxins were identified as the emerging contaminant of concern and a potential threat for fresh water resources. Monitoring microcystin over such large areas is impractical with the current expensive and time consuming method of LC-MS/MS.

Provincially, the province of Quebec is notably affected. In summer, 2011, due to cyanotoxicity, drinking water advisories for public water systems were posted many times by the Quebec Ministry of Sustainable Development, Environment and Parks on their website. Quebec residents were unable to use the municipal water for drinking, bathing or washing purposes. In another incident of Quebec West Island, almost 130,000 people were affected and had no water for the whole summer of 2011 (Bruemmer René, 2011).

FIGURE 5. LAKE ERIE (SOURCE: NASA, 2011)

FIGURE 6. HARMFUL ALGAL BLOOM, LAKE TAIHU, CHINA (GOOGLE IMAGES, 2007)

In the summer of 2011, Lake Erie have seen the largest bloom in decades. The bloom was 22 Km wide (See photo of satellite view- Fig.5).

Internationally, since 1990, blue green algal invasion in Lake Taihu (see satellite photo) the third largest lake of China is causing drinking water crises every summer (Yang Chen, 2003) (Fig.6). In May, 2007, almost two million people were without drinking water for a week due to massive blooms of cyanobacteria in Lake Taihu (Qin *et al.*, 2010).

In view of the global demand for the monitoring of drinking water safety, there is clear research need to prove that ELISA is an effective and efficient method for the detection of microcystins.

Conditions that contributed to the emergence of toxic cyanobacterial bloom as a reoccurring threat include global warming and population explosion. Rise in global temperature created an ecological niche in favour of cyanobacteria over other algae (Parel & Huisman, 2008). Increase in human populations has led to more nutrients loading to the surface water bodies worldwide.

2.7 Response to Reported Blue-Green Algae Events and Microcystins Analysis

Historically, cyanobacterial blooms had been considered a lower risk priority with little environmental consequences. However, due to the widespread of algal blooms over the last few decades and its increased health and environmental risk, now cyanobacteria are identified as one of the emerging contaminant of concern for aquatic systems (CAWQ Symposium, 2012).

Algal bloom incidents have been categorized by the Ministry's Operations Division District Office in priority of "known or anticipated human health impacts", or "known or anticipated environmental impairment" in Ontario. Under this consideration, any cyanobacterial bloom could be potentially toxic and water bodies should be monitored minimum once per week during the peak season of late May to early October (MOE, 2003).

The province's response algorithm to cyanobacterial bloom impinging drinking water supply is summarized in the following flow chart (Fig.7). Under O. Reg. 170/03 water samples are collected by a regulated drinking water system (DWS) and sent to a licensed laboratory for

ELISA testing. ELISA results for total microcystins equal to or greater than 1.5µg/L is considered a provisional exceedance. The licensed ELISA laboratory notifies the MOE and also sends the sample to the Laboratory Services Branch (LaSB) of MOE for the confirmation of microcystin-LR by LC-MS/MS. The results from LC-MS/MS will either confirm or retract the provisional result provided by the ELISA laboratory (MOE, 2010b)

Microcystins-LR results that exceeds (≥1.5µg/) the Ontario Drinking Water Quality Standard (ODWQS) under *O. Reg. 169/03*, are reported to the Ministry of the Environment - Spills Action Centre (MOE-SAC) by the Laboratory Services Branch (LaSB) (Sec.18 *DWA*) (MOE, 2012). Spills Action Centre (SAC) leads the later process; completes the associated Adverse Water Quality Incident (AWQI) number; notify the district Drinking Water (DW) supervisor and AWQI coordinator.

District supervisor/staff contacts the owner/operator of the DWS of concern, the Local Health Unit (OH) and other stakeholders (Conservation Authority, Operation Division (OD), Standards Development Branch, First Nations, Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Ministry of Natural Resources (MNR), Municipalities) to coordinates all other activities until termination of the incident (MOE, 2012).

Sciences & Standards Division (ESSD) of the MOE is also informed to help in providing technical and scientific information (i.e. sampling protocol, presence/absence testing, reporting results), which supports the local Health unit's decision on whether to notify the public, and what measures should be made to safeguard human health. In the MOE, the Drinking Water Management Division (DWMD), specifically the Safe Drinking Water Branch (SDWB), is responsible for any incident related to drinking water-related facilities (MOE, 2012).

All involved groups are responsible for mutual communication on the steps which have been taken (e.g. dissemination of reports coordinated by District office), as well as keeping track of events on the Integrated Divisional System (IDS) (MOE, 2012).

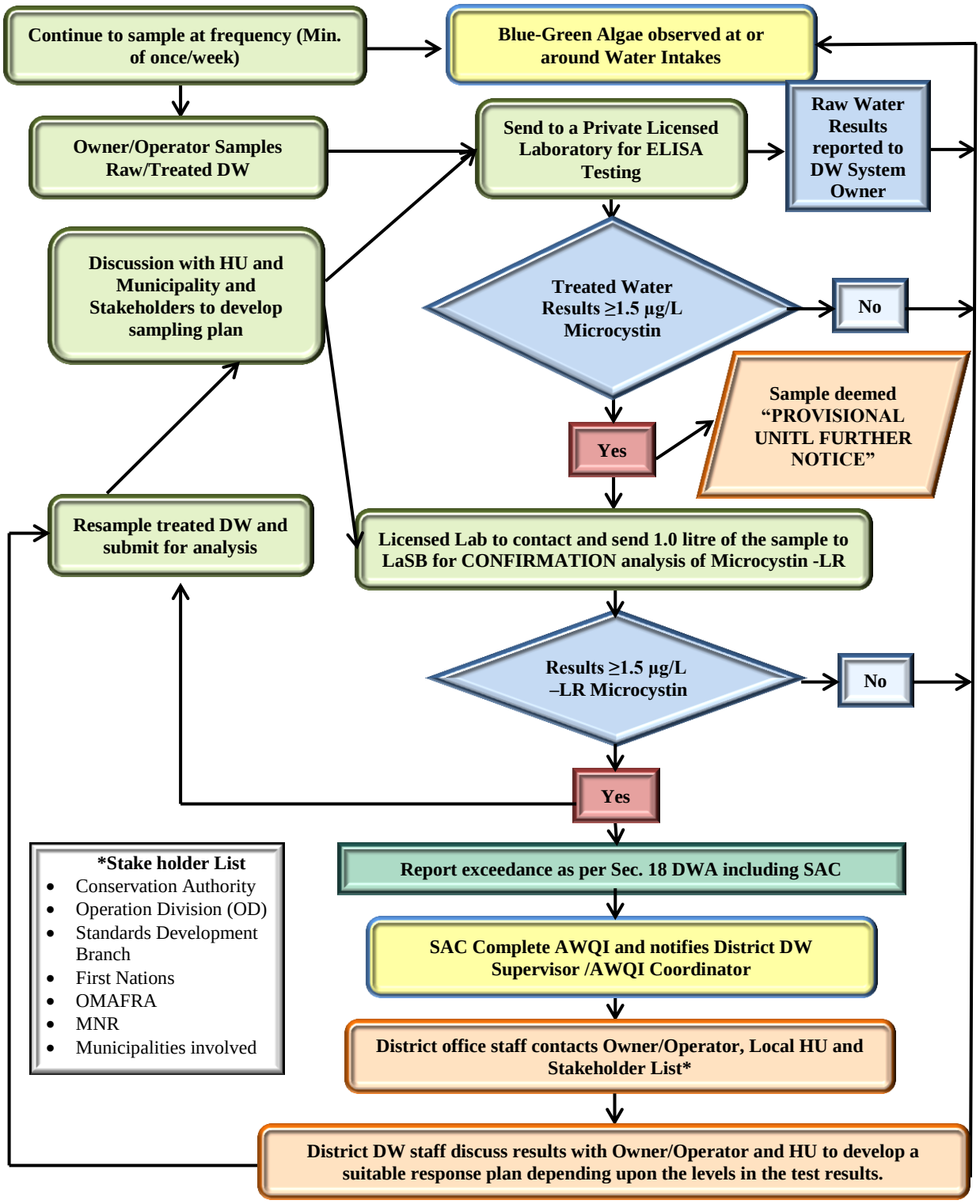

FIGURE 7. FLOW CHART OF RESPONSE TO REPORTED BLUE-GREEN ALGAE EVENTS (SDWB SOP DW.6.02.03.01)

SAC - Spills Action Centre, AWQI - Adverse Water Quality Incident, DW – Drinking Water, HU – Health Unit, OMAFRA - Ontario Ministry of Agriculture, Food and Rural Affairs, MNR - Ministry of Natural Resources *(Source: MOE, DW standards and guidelines, 2010)*

2.8 Regulatory Overview of ELISA and LC-MS/MS

Microcystins was being analysed in the surface and drinking water (raw and treated) by only LC-MS/MS from 2002 to 2009 at Ontario, MOE. On Dec 2009, Ontario Regulation (*O. Reg.*) *248/03* was amended to allow the use of ELISA for drinking water testing (TW). If the result by ELISA shows a potential exceedance (≥1.5µg/L total microcystins) of Ontario Drinking Water Quality Standard (ODWQS), as listed in *O. Reg. 169/03*, then the sample must be analyzed by a licensed method for the confirmation and quantitation of the individual variants, specifically Microcystin-LR. Currently, liquid chromatography - tandem mass spectrometry [LC-MS/MS] is the only approved method for the quantitative analysis of microcystin-LR and the Laboratory Services Branch (LaSB) is the only licensed laboratory to perform it (MOE, 2010a). Laboratories are required to use methods listed in the Protocol of Accepted Drinking Water Testing Methods as amended from time to time (MOE, 2010c).

ELISA: LaSB Method # E3469 (*O. Reg. 248/03*) is an approved screening method for the detection of total microcystins (free and intracellular) in drinking water (MOE, 2010a). The type of immunoassay employed in this method is indirect competitive ELISA (See Section 5.2). The concentrations of the samples are determined by interpolation using the standard curve constructed with each run (MOE, 2010a).

LC-MS/MS: LaSB Method # E3450 (*O. Reg. 248/03*) is an approved method for the quantitative analysis of microcystins variants and Anatoxin-a. The details of the sample preparation and procedure of LC-MS/MS is available in Appendix I (MOE, 2010a).

The above regulatory overview provides the following prospective which is relevant to the focus of this research. Firstly, ELISA and LC-MS/MS are the only two approved methods which are therefore the subjects of scrutiny in this study. Secondly, the regulation was amended for using ELISA to test drinking water, particularly treated drinking water. Raw drinking water source and surface water samples are still being analysed by LC-CMS/MS at MOE because they do not fall under drinking water regulations. Consequently, the full benefit of ELISA has not yet been realized for surface and source water samples analysis. This is the second subject of this research. Finally, the current *SDWA* standard is based on a single toxin variant; but over 90

microcystins variants exist. To address the inadequacy of the current *SDWA* is the third subject in this study.

2.9 Microcystins Analysis

Microcystins can be analysed by multiple methods qualitatively or qualitatively. The selection of the method depends on the purpose of the testing. For example, PCR is an excellent technique to identify the toxin of cyanobacteria, ELISA measures total microcystins while PPIA measures total potential toxicity of microcystins (Fischer *et al*., 2001) and with HPLC, individual microcystins can be separated and recognized on the basis of their retention time (Harada *et al.*, 1996). The advantages and disadvantages of the most common techniques for the analysis of microcystins are summarized in the Table 4.

This study is focused on ELISA and LC-MS/MS because only these two methods are approved for the detection of microcystins in drinking water samples in Ontario. These two techniques (ELISA and LC-MS/MS) will be compared. A third technique, PPIA, will be investigated due to its capability of measuring toxicity directly, an advantageous feature absent from ELISA and LC-MS/MS.

TABLE 4. ADVANTAGES AND DISADVANTAGES OF METHODS FOR THE DETECTION OF MICROCYSTINS

Counter	Method	Advantages	Disadvantages
1	Mouse Bioassay	• Analyse total potential toxicity of sample within hours [1] • Distinguish between hepatotoxins and neurotoxins [1] • Sensitivity at microgram level [1] • Detect toxicity irrespective of the toxin congeners present [3]	• Does not detect toxins at low levels, especially in drinking water [1] • Does not identify specific toxic agent [1] • Low sensitivity; can be used only for the detection of toxin concentrations that could lead to acute toxicity [3] • Intra peritoneal route of administration may not appropriately parallel natural exposures [3] • Many animals and large samples are necessary [3] • Ethical implications [4] • Lack of Reliability [4]
2	ELISA	• No sample prep is required • Allow rapid, easy and effective detection of microcystins [2] • Detection limit is 0.1µg/L • Shows good cross-reactivity with all cyanobacterial cyclic peptide toxin variants [3]	• Does not give any indication of which variants are present in a mixture of microcystins [5] • Can only be used as a semi quantitative screening tool [2] • Toxicity is not assessed [2]
3	HPLC UV (High Performance liquid chromatography ultra violet)	• Can distinguish between microcystins variants provided standards are available [1] • High sensitivity and selective detection [4]	• Does not detect microcystins at levels lower than 1 µg/L [1] • Only a few standards are commercially available [4] • Requires trained personnel, expensive equipment and sample pre-treatment, resulting in long analysis time [4] • Ability to distinguish microcystins is limited as most variants have a similar absorption profile between 200-300 nm [6] • UV detection susceptible to co-eluting compounds that give rise to additional, non-microcystins chromatographic peaks [6]

Counter	Method	Advantages	Disadvantages
4	Reverse Phase HPLC	• Determines numerous variants of microcystins and nodularin [1] • Filtration allows for isolated identification of extracellular and intracellular toxins [1]	• Involves filtration to separate cyanobacterial cells from water [1]
5	MALDI-TOF-MS (matrix assisted laser desorption/ionization-time of flight mass spectrometry)	• Requires only microgram quantities of cell material [2] • Detection is rapidly made without the need of extraction or purification processes [2] • Valuable tool in early warning of toxic bloom formation, enabling rapid detection of whether or not a population contains microcystins-producing genotypes in an early phase of population growth [6]	• Qualitative but not quantitative detection and identification of toxin congeners[9]
6	LC/MS (couple with FAB, ESI, or MALDI-TOF)	• Simple method does not require clean-up because of high selectivity [1] • Enables simultaneous separation and identification of microcystins in a mixture [4]	• Can only analyse –LR, –YR, -LR, -RR at ng/L levels [1] due to limitations of availability of standards
7	Protein Phosphatase Assay (PPIA)	• Sensitive to subnanogram levels in water samples [1] • Many samples quantified in a few hours [1] • Rapid, easy and sensitive; does not require much equipment and is less expensive than ELISA [2]	• Not specific to microcystins, will indicate presence of other substances inhibiting protein phosphatases [1] • Shown to overestimate the toxin concentration [2] • Does not show the same sensitivity for all microcystins variants [7]
8	CE (capillary electrophoresis)	• Toxins can be separated according to differences in their molecular size and charge [7]	• Limited success as it is impeded by adsorption of the analyte onto the inner wall of the capillary [6] • Poor sensitivity compared to HPLC [7]

Counter	Method	Advantages	Disadvantages
9	PCR-based methods	• Can rapidly determine whether a cyanobacterial bloom or species is potentially toxic • QPCR quantifies gene copies (genomic {cell} DNA+eDNA) in cultures as well as in natural samples.[2] • Highly specific for the presence of the *mcyA* component of the microcystins synthetase gene cluster [2]	• Non-quantitative, presence/absence method [8] • Although highly selective for *mcy*A, does not detect microcystins quantitatively [8]

[1](WHO, *2003*, pg.8-9,) [2](de Figueiredo *et al.*, 2004), [3](Fischer *et al.*, 2001), [4](Campas *et al.*, 2007) [5](Mountfort *et al.*, 2005), [6](Sangolkar *et al.*, 2006), [7](McElhiney & Lawton, 2005*),* [8] (Hawkins *et al.*, 2005), [9](Howard *et al.*, 2007)

2.9.1 Liquid Chromatography – (Electrospray Ionization) Tandem Mass Spectrometry [LC-(ESI) MS/MS.

LC(ESI)-MS/MS is an analytical chemistry technique that combines the physical separation capabilities of high pressure liquid chromatography (HPLC) with the mass analysis capabilities of tandem mass spectrometry (Li *et al.*, 2006). Gramicidin S is used as the internal standard (MOE, 2010a). Microcystin-LR, -RR, -LA, -YR and anatoxin-a are determined quantitatively by multi-point calibration. This method enables simultaneous separation, identification and quantification of microcystins variants in a mixture (Bruno *et al.*, 2006).

However, identification of variants are limited due to overlapping molecular weights, retention time and availability of few commercial reference standards (-LR, -RR, -LA, -YR) (Hotto, 2007; Fisher *et al.*, 2001). It is very time consuming and expensive (Fisher *et al.*, 2001). Consequently, it cannot measure all 90+ microcystins variants that might be present in the water. The detailed procedure of LC-MS/MS is available in Appendix I.

2.9.1.1 Matrix and Matrix Interference

In environmental analytical chemistry, the type of sample obtained from different environments contains different constitutes. For example, surface water, untreated source water and treated drinking water have entirely different chemical constituents; the first one may be rich in biomass, the second one has no chlorine but third one is filtered and chlorinated. These three types of water would be considered as three different matrices in which the analyte (microcystins) is sought after.

Matrix interference refers to the components of sample other than the target analyte that interfere with the test result such that reliable data cannot be generated. Examples of matrix interference include samples with extreme pH, chemical constituents that react with target analyte, and sludgy samples or samples over-saturated with biomass. Since the exact component which causes unreliable results is usually undetermined, such undesirable effects on the result due to a particular matrix are generally referred to as matrix effect.

Microcystins are analysed by liquid chromatography (LC)-multiple reaction monitoring (MRM) tandem mass spectrometry (MS/MS) on a triple quadrupole mass spectrometer at the LaSB of MOE. Method#3450 is an approved method for the analysis of microcystin-LR by MOE. A variety of matrices can affect the analysis of LC-MS/MS. Chemical interferences, that are not resolved by chromatography or the unit mass resolution of the tandem quadrupoles, may be present in some samples. These samples may require additional selectivity via additional sample clean-up and/or higher resolution MS/MS or MS/MS/MS, high resolution mass spectrometry (HRMS) or Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS).

The sample preparation method is restricted to water samples. Applicability of the method to samples with very high organic content, such as effluents and water containing high concentrations of humic materials, is unknown.

Algal toxin stability is possibly matrix dependent. The presence of microbes, humic material and residual chlorine may contribute to compound losses (MOE SOP#3450-2012).

2.9.2 ELISA (Enzyme-Linked Immunosorbent Assay)

Enzyme-linked immunosorbent assay (ELISA) is a biochemical technique that involves antibodies and enzyme for the detection of microcystins in a sample. Most commonly used techniques are based on two principles: direct competitive ELISA and indirect competitive ELISA.

Direct competitive ELISA is based on the principle that microcystin-LR in the sample compete with microcystin-LR-peroxidase for the limited number of binding sites of anti-microcystins antibody attached to the microtiter plate. The strength of the colour development is inversely proportional to the concentration of microcystins (Carmichael & An, 1999). In an indirect competitive ELISA, microcystins present in sample and a monoclonal antibody against microcystin-LR compete for the binding sites on a microcystin-LR-Bovine Serum Albumin (BSA) coated plate. A secondary antibody conjugate (HRP conjugated goat anti-mouse IgG) is added and finally addition of substrate produces color. The intensity of the color is inversely proportional to the concentration of the microcystins present in the sample (Carmichael & An, 1999). The results by immunoassays are compared to a standard curve with the known concentrations. Microcystin-LR is used as a calibrating agent and the amount of microcystins can be reported as microcystins-LR equivalents (Harda *et al.,* 1999).

Specificity refers to the ability of an individual antibody or enzyme to react with only one antigen or substrate, respectively (Mayer, 2010). Antibody specificity measures the degree to which the antibody differentiates between different antigens. Cross-reactivity measures the extent to which different antigens appear similar to the antibody (Frank SA, 2002). Sensitivity is the ability to recognize and bind to antigen. In practical terms, the sensitivity of an ELISA is described by the method detection limit (MDL).

Antibodies used in ELISA can be polyclonal (Fischer *et al*., 2001; An & Carmichael *et al*., 1994; McDermott *et al.,* 1995) or monoclonal (Zech *et al.,* 2001) produced against microcystin-LR. Finding a reliable method to detect multiple variants of microcystins in a single sample has always been a challenge. Current anti-ADDA technology can detect multiple variants of microcystins with acceptable sensitivity. ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-

phenyldeca-4,6-dienoic acid) component of microcystins is present in > 80% of known toxic variants of microcystins (Metcalf *et al.,* 2001) and nodularins. Polyclonal antibodies as compare to monoclonal antibodies react with more microcystins variants and nodularin (Metcalf *et al.,* 2001). Polyclonal-ADDA-ELISA slightly overestimates the total microcystins due to recognition of the free ADDA moiety (Metcalfl *et al.,* 2001) which is non-toxic but could possibly be present in occasional environmental samples.

ADDA moiety can be detected by monoclonal-ADDA-ELISA (Zech *et al.,* 2001). However, the cross reactivity of monoclonal antibodies with variants other than -LR is weaker or absent, compare to the polyclonal antibody to ADDA. Monoclonal-ADDA-ELISA might underestimate the sum of microcystins congeners of microcystins (Abraxis, 2008).

Polyclonal-ADDA-ELISA kit by Abraxis is used at MOE to capture as many variants as possible in order to access the magnitude of potential health risk. This kit is based on the indirect competitive ELISA principle. A brief summary of cross reactivity of ELISA antibodies with variants by Abraxis (manufacturer) versus few other researches has been summarized the Table 5.

The antibodies against microcystins are detected by the enzyme labelled (Horse redish peroxidase (HRP), alkaline phosphatase or fluorescence labeled) to them. Enzyme produce colored signal which are measured with spectrophotometer. The most commonly used enzyme in ELISA is horse reddish Peroxidase (HRP) because of its smaller size which reduce steric hindrance of the antibody moleclue (Azevedo *et al.,* 2003). Peroxidase is also economical, rapid and a more stable enzyme than the other enzymes.

TABLE 5. MICROCYSTINS CROSS-REACTIVITY COMPARISON BETWEEN MULTIPLE STUDIES

Variants	ADDA Abraxis (2008)	Sheng *et al*, (2006)	Fischer *et al*., (2001	Metcalf *et al*, 2000	Code *et al* , 1989
Manufacturer	Abraxis	Developed in house	Developed in house	Developed in house	Commercially available kit (unknown)
Antibodies	Anti ADDA antibodies	Polyclonal anti-mcyst-LR antibodies	Anti ADDA antibodies	Polyclonal anti-mcyst-LR antibodies	Polyclonal anti-mcyst-LR antibodies
ELISA Technique	Indirect competitive ELISA	Direct competitive ELISA	Indirect competitive ELISA	Indirect competitive ELISA	Unknown
Unit of measurement	Cross reactivity w/w	Cross reactivity % relative to mcyst-LR	Cross reactivity % relative to mcyst-LR	50% B_0 concentration (µg/L)	50% B_0 concentration (µg/L)
Microcystin-LR	100	100	100	2.5	0.32
Microcystin-RR	91	177 ± 21	50	-	0.36
Microcystin-YR	78	97 ± 16	167	-	-
Microcystin-LA	125	-	-	2	-
Microcystin-LW	114	69 ± 13	118	5	0.71
Microcystin-LF	108	77 ± 15	108	2.7	1.2
Microcystin-LY	-	-	-	2	0.56
Nodularins	169	63 ± 15	100	5.7	0.36
dmMC-LR	158	-	157	-	-
dmMC-RR	77	-	80	-	-

w/w = weight/weight basis, 50% B_0 values represent binding of 50% of the antibodies in the indirect competitive ELISA

2.9.3 Protein Phosphatase Inhibition Assay (PPIA)

Phosphatase inhibition activity of microcystins can be detected in water samples directly using an enzyme protein phosphatase 2A and a substrate *p*-nitrophenyl phosphate in a colorimetric phosphatase inhibition assay (MacKintosh *et al.*, 1990). Sample is incubated with protein phosphatase enzymes 1A or 2A. Microcystins present in the sample binds to the protein phosphatase. Dephosphorylation of *p*-nitrophenyl phosphate (substrate) by protein phosphatase

to *p*-nitrophenol at high pH produces a yellow color which is measured with spectrophotometer (405nm absorbance). The strength of this color is inversely proportional to the concentration of microcystins present in the sample (Carmichael & An, 1999). Although assays have been developed for both PP1 and PP2A, the reaction of microcystins with PP1 is around 50 times less sensitive than with PP2A (McElhiney & Lawton, 2005) and so is not as popularly used.

The limitations of PPIA are, it is not specific to microcystins, other non-microcystins protein phosphatase inhibitors are detectable too, (Albay *et al.*, 2003) such as okadeic acid and not all the microcystins variants react with protein phosphatase enzymes to a similar extent (Sangolkar *et al.*, 2006).

An advantage of PPIA over ELISA is its ability to detect bioactivity of microcystins, rather than limited recognition of a structural component (Carmichael & An, 1999). The affinity of microcystins for protein phosphatases is particularly high, such that very low levels of toxin are detectable (Sim & Mudge, 1993).

2.10 Comparison of ELISA, LC-MS/MS and PPIA

LC-MS/MS, ELISA and PPIA have their own advantages and limitations for the detection of microcystins. Hence, no single technique is sufficient in providing both a precise measurement of toxicity and an accurate profile of the microcystins variants (McElhiney & Lawton, 2005). ELISA can detect total microcystins, PPIA measures the toxicity and LC-MS/MS can identify four individual variants (-LR, -YR, -RR and -LA). A literature review on the historic development and comparisons of these three analytical methods are summarized below.

McDermott *et al,* (1995) generated antibodies against microcystin-LR and designed an ELISA. They reported an agreement between ELISA and HPLC results.

Ward *et al,* (1997) reported that colorimetric protein phosphatase inhibition assay can underestimate microcystins at concentration lower than 0.8ng/mg dry wt. They found a good correlation with $R^2 > 0.93$ and $p < 0.0001$ between PPIA and HPLC, and recommended PPIA as a suitable method for screening the microcystins.

Rivasseau *et al,* (1999) compared the results of microcystins from a commercially available ELISA kit (unnamed) with the results obtained from solid phase extraction followed by liquid chromatography (n=8, r^2=0.989. The detection limit was 0.1 to 0.15µg/L in ground water and surface water. The kit proved to be capable of displaying reproducibility and accuracy in analyzing environmental water samples for the detection of microcystins. Cross reactivity among some microcystins variants was observed.

Carmichael & An (1999) reported that ELISA and PPIA are less quantitative than the physicochemical assays but they are just as sensitive, more rapid and useful in the screening of environmental samples. He suggested that ELISA technique can be used successfully for routine screening of water for microcystins contamination (Msagati *et al.*, 2006; Metcalf *et al.*, 2002).

Tsutsumi *et al,* (2000) compared sandwich type of ELISA with the direct ELISA. Sandwich ELISA was found to be more sensitive with a detection limit of 2 to 100 pg/mL (ng/L or ppt) and also showed a good cross reactivity towards -RR and -YR variants of microcystins.

Lawrence & James *et al*, (2001) compared microcystins results by ELISA, PPIA and LC-MS/MS in more than 100 blue-green algae products in the form of pills, capsules, and powders. The samples were extracted with 75% methanol in water and centrifuged to remove solids before analysis. The results obtained by ELISA and PPIA agreed well with LC-MS/MS over a concentration range of about 0.5-35µg/g. However, research paper does not explain the selection criteria of certain analytical technique for a chosen sample because not all the samples were tested by three methods. Research paper is also not clear about the performance of agreement calculation among three methods.

Zeck, Anne *et al,* (2001) developed a direct competitive ELISA with a monoclonal antibody (clone (MC10E7). Microcystin-LR spiked samples in the concentration range between 0.01 and 0.1µg/L were measured and a mean recovery of 99.9±16.4% was achieved. The detection limit for microcystin-LR was 6ng/L (ppt). Antibodies used in this assay were also tested for its robustness against interference from humic acids, pH, salt content, surfactants or organic

solvents. The antibodies were found to be very stable. The assay was found to be extraordinary sensitive and highly selective for microcystin-LR analysis in drinking and surface water samples.

Metcalf *et al*, (2001) developed a PPIA to overcome the specificity problem of PPIA inhibition to not only microcystins and nodularins but also other inhibitors such as okadiac acid, calyculin A and tautomycin. They combined the immunoassay based detection of the toxins with a colorimetric protein phosphatase inhibition system in a single assay, designated the colorimetric immuno-protein phosphatase inhibition assay (CIPPIA). Samples were incubated with anti-LR antibodies which will neutralize any microcystins if present. Subsequent addition of protein phosphatase will produce an enzyme reaction because the antibodies protected the enzyme from the inhibition by microcystins. CIPPIA was quantifiable by calculating a protective index based on the colorimetric enzyme reaction relative to controls using microcystin-LR standard, immune serum and pre-immune serum. The protective index values distinguished seven purified microcystins variants (microcystin-LR, -D-Asp3-RR, -LA, -LF, -LY, -LW, and -YR) and nodularin from okadaic acid, calyculin A, and tautomycin. CIPPIA showed a good correlation (R^2=0.94, P<0.00001) with high-performance liquid chromatography.

Fischer *et al.*, (2001) raised antibodies to ADDA and developed an indirect competitive ELISA. The MDL of the assay was 0.02-0.07 ng/mL (µg/L) for drinking water and surface water. ELISA was found to be more sensitive (5-folds) than PPIA for the detection of microcystins in the surface water. He recommended that ADDA-based ELISA is a powerful tool to detect the numerous variants of microcystins and nodularins at the levels below the WHO guidelines (1.0µg/L) for drinking water without any sample preparation.

Heresztyn and Nicolson *et al.*, (2001) developed a colorimetric phosphatase inhibition assay using protein phosphatase enzyme type 2 subunit A (PP2A) and p-nitro phenyl phosphate as substrate for the detection of microcystins. The assay was comparable with radiolabelled substrate phosphatase inhibition assay with working range of 0.2–1µg/L. The assay was robust and was not affected by matrix interference.

Bouaïcha *et al.,* (2002) compared colorimetric and fluorometric protein phosphatase inhibition assay for the detection of cyanobacterial peptide hepatotoxins in drinking water. The colorimetric assay was equally sensitive to fluorometric method with the detection limit range of 0.25 to 0.1µg/L. Colorimetric PPIA method does not provide detailed information on the chemical identity of the inhibitors present in the water samples. However, it was found to be an easy and economical screening tool to detect the toxic microcystins variants to reduce the number of water samples that may require further analyses by other more expensive and elaborate method.

Rapala *et al.,* (2002) compared colorimetric PPIA, a commercial ELISA test and HPLC methods for the detection of cyanobacterial hepatotoxins; microcystins (MCYST) and nodularin. Concentrations of microcystins variants detected by ELISA were lower than the other methods (PPIA & HPLC). However, it was found that ELISA can accurately detect the presence or absence of total microcystins and PPIA can accurately detect the toxicity.

Michael & Greogry (2002) compared PPIA and ELISA. The similarity Index (SI) of 86% was found between PPIA and ELISA (Table 6).

TABLE 6. A COMPARISON OF ELISA AND PPIA METHODS FOR CLASSIFYING SAMPLES INTO DIFFERENT TOXIN LEVEL CATEGORIES

Category (µg/L)	**$PPIA_a$**	**$ELISA_b$**	**$Both_{ab}$**	**Similarity Index (SI) (%)**
Less than 0.2	42	45	42	97%
0.2–0.6	19	20	16	82%
0.6–1.0	8	3	1	23%
1.0–5.0	16	17	12	73%
5.0–10.0	1	1	1	100%
10.0–100	4	4	4	100%
Greater than 100	9	9	9	100%
Total	99	99	85	86%

SI(%) was calculated as $SI = (n_{ab} /2) \times (1/n_a + 1/n_b)$ where nab is the number of samples classified the same by both ELISA and PPIA, and n_a is the number classified by PPIA, and n_b the number classified by ELISA.
Source: Satchwell and Boyer (2002)

Metcalf *et al.,* (2003) found the mean recovery efficiency of ELISA was between 99% and 101% for the evaluation of microcystins in the water. False positive results were produced in the ample

spiked with sodium chloride. The findings are critical for the analysis of microcystins in the brackish water.

Mathys & Surholt (2004) compared HPLC and ELISA. Microcystins was analysed by using a commercially available ELISA kit (EnviroGardTM, Germany). They made some modifications to the ELISA protocol by modifying incubation time and temperature. The modified ELISA results agreed very closely to the sum of variants of microcystin -YR, -RR, and -RR tested by HPLC.

Hawkins *et al.,* (2005) analysed cyanobacterial cells by microscopy and microcystins by mouse bioassay, ELISA, PCR, PPIA, HPLC and LC-MS/MS. The performance of each method was measured on the basis of microcystins detection limit, cost, level of analyst training required, selectivity for microcystins, and turnaround time. ELISA was ranked first due to its advantages. PPIA and ELISA together were found to be the methods of choices to detect toxic microcystins accurately. PCR was found to be highly sensitive and not costly either, but its use was recommended in a combined assay format. HPLC and microscopy were ranked in the mid-range. Microscopy was cheaper but not recommended due to false results. LC-MS/MS was found to be very expensive. The mouse assay was excluded due to its sensitivity, which was below the WHO drinking water guideline (1.0µg/L).

Hilborn *et al,* (2005) used a commercial ELISA (EnviroLogix, Inc., Portland, ME, USA) to analyse microcystins in human serum and compared the results with LC-MS/MS. The spearman correlation was calculated between the two methods. A good correlation (Spearman $r = 0.96$, $p < 0.0001$) was found. ELISA was concluded as a reliable and affordable assay to screen microcystins in human sera.

Pyo *et al,* (2005) developed a competitive enzyme-linked Immunosorbent assay (ELISA) based on monoclonal antibodies against microcystins -LR. The ELISA was highly sensitive and suitable for the trace analysis of microcystins in water. The linear response between monoclonal antibodies with different concentrations of microcystin-LR was established between 30 and 1600pg/mL (ng/L or ppt).

Sheng *et al,* (2006) generated polyclonal antibodies by immunization of MC-LR-BSA (microcystin-LR conjugated to bovine serum albumin) for a direct competitive ELISA to detect microcystins in water. The assay showed a good cross-reactivity with microcystin-variants (-LR, -RR, -YR, -LF, -LW) and nodularin. The detection limit for microcystin-LR was 0.12µg/L. The recovery of microcystins from spiked samples by ELISA was 90 to 110%.

Bruno *et al,* (2006) used LC-MS/MS to measure the recovery of four microcystins-variants (-LR, -YR, -LA, -RR) in food supplements and compared the recovery of total microcystins by ELISA. In a t-test, significant difference was observed between ELISA and LC-MS/MS ($t = 0.00215$, $p<0.05$) in pills but not in capsules ($t = 0.247202$, $p<0.05$). They speculated that the underestimation of microcystins by ELISA was possibly due its different sensitivities for some toxic variants.

Sangolkar *et al,* (2006) in their review suggested that bioassays like ELISA and PPIA provide enough information regarding presence or absence of toxin. Hence, they can be used to screen microcystins in the water. Samples positive by these methods can be further analysed for qualitatively and quantitatively for the variants by HPLC coupled to photodiode array (PDA) detector or MS.

Tillmans *et al,* (2007) reported that ELISA and HPLC produced similar results for microcystin-LR standards. However, concentration of microcystins detected by ELISA was found to be four times higher than HPLC in environmental samples. The difference may depend on number of variants in situ and number of microcystins that can be identified by HPLC.

Ikehara *et al,* (2008) expressed a catalytic subunit A of the recombinant protein phosphatase (rPP2A) in a baculovirus system. The rPP2A enzyme was then used in a microplate PPIA. This version of PPIA using a recombinant enzyme subunit is referred to as PP2A. They reported that PP2A was able to detect microcystins concentrations from 0.005 to 5ng/mL (ppt) in fresh water. Pre-treatment of water samples was not necessary. This microplate assay was more sensitive than ELISA. Hence, rPP2A based PPIA was found to be an excellent tool for detecting and quantifying the toxicity in water.

Geis-Asteggiante *et al,* (2011) used multiple samples extraction methods of fish for the analysis of microcystins and nodularins. The samples were analysed by ELISA and LC-MS/MS. The comparison indicated that ELISA was unable to distinguish between microcystins variants but correctly assessed the presence or absence of microcystins and nodularin-R in the fish tissues.

Sassolas, Audrey, *et al,* (2011) developed a colorimetric phosphatase inhibition assay for the detection of microcystin-LR. He tested microcystins with a molecularly engineered PP1 and a commercial PP2As (Millipore, ZEU Immunotec). To overcome the instability problem of protein phosphatase enzymes, both PP2A and PP1 were entrapped on agarose gel. Results based on PP2A from ZEU Immunotec immobilised showed the best performances with a detection limit of 0.17µg/L and PP1 (molecularly engineered) showed a detection limit of 0.28µg/L. These results demonstrated that both assays can detect concentration of microcystin-LR lower than the maximum than the WHO guideline (1.0µg/L).

Pírez, Macarena *et al,* (2013) used a commercial ELISA (Alexis Biochemicals, San Diego, CA, USA) for the detection of microcystins to establish a sustainable monitoring program for recreational water. The ELISA was able to detect 1000-fold higher microcystins than the WHO limit (20µg/L for moderate risk) for recreational water. ELISA results demonstrated that cyanobacterial cell counts and chlorophyll-a (parameters for indirect estimation of toxicity) were poor indicators that can be highly misleading. ELISA proved to be more economical, rapid and reliable for the monitoring of microcystins in the water.

TABLE 7. LITERATURE REVIEW SUMMARY OF ELISA, PPIA AND LC-MS/MS METHODS FOR MICROCYSTINS IN TERM OF DETECTIONS

Group #	Estimation of microcystins by ELISA	# of reports	References	Comments
1	Overestimates	5	Rivasseau *et al.,* 1999	ELISA slightly over estimated microcystins when compared with LC-MS/MS
			Tsutsumi *et al.,* 2000	ELISA overestimate
			Conti *et al.,* 2005	ELISA results were always higher than LC-MS/MS
			Mountfort *et al.,* 2005	Elisa overestimate
			Tillmans *et al.,* 2007	ELISA concentrations were found to be four times greater than HPLC
2	Detects accurately	16	McDermott *et al.,* 1995	An agreement between ELISA and HPLC results of microcystin-LR
			Carmichael & An, 1999	ELISA and PPIA less quantitative but equally sensitive
			Tsutsumi *et al.,* 2000	Very specific for microcystins
			Lawrence & James, 2001	ELISA results well agreed with LC-MS/MS within the range of 0.5 to 35µg/L of microcystins in water
			Fischer *et al.,* 2001	ELISA is very sensitive for microcystins variants and also for Nodularins
			Zeck *et al.,* 2001	Mean recovery of microcystin-LR by ELISA was 99.9±16.4%
			Metcalf *et al.,* 2003	Mean recovery efficiency of microcystins was 99% to 101% but produced false positive results in the presence of NaCl
			Rapala *et al.,* 2002	ELISA is equally sensitive to HPLC in lower concentration of microcystins
			Mathys & Surholt, 2004	Sum of -LR, -YR, -RR, and -RR by ELISA and HPLC were equal
			Mountfort *et al.,* 2005	ELISA produced higher results than LC-MS/MS
			Hawkins *et al.,* 2005	ELISA is equally sensitive to HPLC

Group #	Estimation of Microcystins by ELISA	# of reports	References	Comments
			Pyo *et al.*, 2005	Competitive enzyme-linked immunosorbent assay (ELISA) based on monoclonal antibodies against (microcystin-LR) is highly sensitive for the trace analysis of cyanobacterial hepatotoxins
			Sangolkar *et al.*, 2006	ELISA detect accurately
			Sheng *et al.*, 2006	Established an enzyme-linked immunosorbent assay (dc-ELISA) to detect the microcystins in waters, which showed a good cross-reactivity with microcystin - LR, RR, -YR, -LF, -LW and nodularin. A good recovery was found.
			Geis-Asteggiante Lucía *et al.*, 2011	ELISA was unable to distinguish between microcystins but was found to correctly assess the presence or absence of microcystins and Nodularins
			Pírez, Macarena *et al.*, 2013	ELISA accurately detect and a method of choice for monitoring microcystins
3	Underestimate	1	Bruno *et al.*, 2006	ELISA underestimate microcystins when compared with LC-MS/MS

The entire literature review is summarized in Table 7, which showed two dominant groups; Group 1 in which ELISA overestimates microcystins and Group 2 where ELISA detects accurately. The reason for Group 1 could be the presence of more microcystins variants which other techniques (HPLC and LC-MS/MS) cannot detect due to limited standards. Group 2, could represent study sample that contained mainly those microcystin variants which are detectable by LC-MS/MS. Thus, literature review leads to a clear conclusion that sensitivity of ELISA is equal to or greater than LC-MS/MS for the detection of total microcystin concentration.

Although ELISA reports total microcystins, it does not provide toxicity information. The current MOE drinking water guideline of 1.5µg/L is based on the toxicity of the microcystin-LR variant. At similar concentrations, toxicity due to other variant or mixture of variants would be different

from that of microcystin-LR. Hence, a toxicity assay is desirable. Five articles (Fischer *et al.*, 2001; Sim & Mudge, 1993; Sassolas, Audrey *et al*., 2011; Bouaïcha *et al.*, 2002; Heresztyn & Nicolson, 2001) agreed that colorimetric PPIA can detect the total toxicity of microcystins without any matrix interference (Heresztyn & Nicolson, 2001). This was further confirmed by six more studies (Ward, Clive *et al.*, 1997; Rapala *et al.*, 2002; Robillot & Hennion, 2004; Sangolkar *et al.*, 2006) that found good agreements between PPIA and HPLC, and PPIA and LC-MS/MS (Lawrence & James, 2001). However, PPIA is not specific to microcystins. It can also detect other non-microcystins, protein phosphatase inhibitors such as okadeic acid and calyculin A (Albay *et al.*, 2003; Lin *et al.*, 1994; Metcalf *et al.*, 2000).

In summary, literature review very clearly indicates that use of ELISA and PPIA together can provide enough information to protect the public from microcystins toxicity (Mountfort *et al*., 2005; Carmichael & An, 1999, Rapala *et al*., 2002; Lawrence & James, 2001; Ikehara *et al*., 2008) and more meaningful regulations for drinking water can be established based on these two assays.

3 Agreement between ELISA and LC-MS/MS

Microcystins are analyzed at Laboratory Services Branch (LaSB) of Ontario Ministry of the Environment (MOE) by ELISA and LC-MS/MS. The data was retrieved through Laboratory information system (LIMS) of MOE from 210 to 2012. This chapter will cover in detail the material, method, agreement calculation between ELSIA and LC-MS/MS results, summary and discussion. The details of agreement calculations are as follow:

3.1 Material and Method

Microcystins test results by both methods (ELISA and LC-MS/MS) were collected from MOE data base (2010 to 2012).The microcystins test results will be organized in two separate and distinct categories from each year for good reasons. The two categories are: drinking water (WD) and surface water (WS). One of the reasons for doing so is because drinking water testing falls under the regulations governed by the *Safe Drinking Water Act* (*SDWA*) 2002. On the contrary, surface water usually represents recreational water from beaches or rivers; the testing of these samples is not regulated under any legislation. Another reason is that the Method Detection Limit (MDL) depends on matrix effect. Raw or drinking water, before or after water treatment plant processing respectively, is relatively clear from matrix effect. Consequently MDLs are achievable by both ELISA and LC-MS/MS methods. On the contrary, WS usually contain debris and impurities that may exert matrix effect to various extents. In the case of ELISA this matrix effect could be eliminated by clarifying the water samples simply by centrifugation. This is not the case with LC-MS/MS method, where values of microcystins has to be raised sometimes by 50-fold in order to disregard the noise.

Once, the data organized into two types of matrices (WS, WD) from each year, microcystins values by both methods will be compared and sorted depending upon the MDL (ELISA 0.15 µg/L , LC- MS/MS 0.05 µg/L) of each and will be placed in 4 Tables: (1) samples positive by both methods (ELISA and LC- MS/MS) (2) samples positive by ELISA and negative by LC-MS/MS (3) samples negative by ELISA and positive by LC-MS/MS (4) samples negative by both methods (ELISA and LC- MS/MS). The results higher than MDL will be highlighted in **red color** and lower or equal to MDL in **green color**.

3.1.1 Calculation of Agreement

The number of samples will be counted from 4 Tables individually and will be organized in Table 8 for agreement calculation between ELISA and LC- MS/MS.

a = Samples tested positive by both methods

b = Samples tested positive by ELISA and negative by LC-MS/MS

c = Samples tested negative by ELISA and positive by LC-MS/MS

d = Samples tested negative by both methods

TABLE 8. AGREEMENT CALCULATION BETWEEN ELISA AND LC-MS/MS

Year of Test Results	**LC-MS/MS Tested**			**Total**
		Positive	**Negative**	
ELISA Tested	**Positive**	a	b	
	Negative	c	d	
				Total Samples
Agreement (%) = (a+d)/(a+b+c+d)				

3.1.2 Regression Analysis

Scattered plot will be performed to find out whether there is any correlation exists between ELISA and LC-MS/MS values. LC-MS/MS will be plotted in x-axis and ELISA in y-axis. A trend line will be plotted and linear regression value (R^2) will be calculated.

3.2 2010 Agreement Calculation between ELISA and LC-MS/MS

Microcystins samples tested by both ELISA and LC-MS/MS in 2010 are sorted on matrix type in two groups; WS and WD. These two groups are discussed individually and then summarized collectively in one table for the agreement calculation of whole year (2010). The details of the agreement calculations are as follow:

3.2.1 2010 Surface Water (WS) Results – Agreement between ELISA and LC-MS/MS

In 2010, ELISA and LC-MS/MS test results were available on 77 surface water samples. These 77 samples are sorted in Table 9 to 11 and Table A1 according to agreement between ELISA and LC-MS/MS results. Where there was disagreement, due diligence was given to consider the results in terms of (1) test reliability and (2) impact on environmental and public health.

3.2.1.1 ELISA Positive and LC-MS/MS Negative Samples

Table 9 depicts five samples which were found positive by ELISA and negative by LC-MS/MS. The normal MDL of LC-MS/MS is 0.05µg/L in the absence of matrix interference, which was the case in counter #1 to #3. In these three cases, the quantities of microcystins detected by ELISA are well below Ontario Drinking Water Guidelines (1.5µg/L) therefore imparting no health threat. It is possible that ELISA were detecting variants other than those four analyzed by LC-MS/MS.

TABLE 9. 2010 SURFACE WATER SAMPLES (WS) THAT WERE ELISA POSITIVE AND LC-MS/MS NEGATIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C180932-0001	WS	0.05	0.05	0.05	0.05	0.02	0.2
2	C180126-0001	WS	0.05	0.05	0.05	0.05	0.02	0.3
3	C178947-0004	WS	0.05	0.05	0.05	0.05	0.02	0.7
4	C182002-0001	WS	0.5	0.5	0.5	0.5	0.2	0.41
5	C178947-0001	WS	1.0	2.0	2.0	2.0	0.5	2.8
Total 2010 WS samples = 5								

Counter #5 describes a sample with noticeable matrix interference. In this case, the MDL of the LC-MS/MS has to be raised 40-fold from 0.05 to 2.0µg/L in order to disregard the background noise. As a result, the 2.8µg/L microcystins detected by ELISA had escaped by LC-MS/MS detection. Since this quantity exceeds the Ontario Drinking Water Guidelines, this surface water poses a health threat especially to wild life.

Counter #4 describes a scenario similar to but less severe than Counter #5. In this case, the matrix interference prompted a 10-fold MDL elevation and subsequent non-detect for variants by LC-MS/MS. The 0.41µg/L quantity detected by ELISA poses an acceptable health risk. Counter #4 could be viewed as a continuum between Counter #1 to #5.

Samples in Table 9 are excellent candidates if available for PPIA analysis in order to assess potential toxicity.

3.2.1.2 ELISA Negative and LC-MS/MS Positive Samples

Table 10 represents the samples found positive by LC-MS/MS and negative by ELISA. Values from counters #1 to #7, #10, #11 are less than WHO threshold (1.0µg/L) by both methods. LC-MS/MS in counter #8, #9 #12, and #13 have values higher or equal to *SDWA* threshold but negative by ELISA. These four samples pose a significant threat to wild animals and environment. However, it is very strange that in counter #9 all values by LC-MS/MS are identical and MDL by LC-MS/MS had been raised 5-folds which make the results of this sample questionable.

TABLE 10. 2010 SURFACE WATER SAMPLES (WS) THAT WERE ELISA NEGATIVE AND LC-MS/MS POSITIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C177701-0001	WS	0.05	0.05	0.05	0.11	0.11	0.02	0.15
2	C179357-0002	WS	0.05	0.05	0.05	0.35	0.35	0.02	0.15
3	C179958-0002	WS	0.05	0.05	0.05	0.19	0.19	0.02	0.15
4	C180745-0003	WS	0.05	0.05	0.05	0.07	0.07	0.02	0.15
5	C179492-0001	WS	0.05	0.05	0.05	0.09	0.09	0.02	Not Detected
6	C179492-0006	WS	0.05	0.05	0.05	0.11	0.11	0.02	Not Detected
7	C179540-0002	WS	0.05	0.05	0.05	0.1	0.1	0.02	Not Detected
8	C179589-0001	WS	0.05	4.1	0.05	0.29	4.39	0.46	Not Detected
9	C179406-0001	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
10	C179492-0002	WS	0.25	0.05	0.05	0.31	0.56	0.02	Not Detected
11	C179492-0005	WS	0.27	0.05	0.05	0.29	0.56	0.02	Not Detected
12	C178878-0001	WS	1.1	0.05	0.05	5.3	6.4	0.02	Not Detected
13	C179089-0001	WS	27	0.33	0.38	14	41.71	2.0	Not Detected
Total 2010 WS samples = 13									

At first glance on the Table 10, one common element can be observed that -LA variant of microcystins is positive by LC-MS/MS for all the samples. Perhaps the antibodies from Abraxis (2009b) kit are not specific against -LA but this phenomenon is no longer observed in the following years (2011-2012).

3.2.1.3 Samples Positive by both ELISA and LC-MS/MS

Table 11 exhibits a good agreement between LC-MS/MS and ELISA samples from counter #1 to #16. However, all ELISA values are slightly higher than LC-MS/MS. This trend suggests the

hypothesis that ELISA can detect more variants of microcystins than LC-MS/MS. This hypothesis can be tested using PPIA to assess toxicity and potential health concerns.

TABLE 11. 2010 SURFACE WATER SAMPLES (WS) THAT WERE POSITIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C179324-0001	WS	0.05	0.05	0.05	0.11	0.11	0.04	0.25
2	C179958-0001	WS	0.05	0.05	0.05	0.19	0.19	0.02	0.35
3	C179982-0004	WS	0.06	0.05	0.05	0.31	0.37	0.02	0.4
4	C179540-0001	WS	0.05	0.05	0.05	0.2	0.2	0.02	0.5
5	C180253-0002	WS	0.11	0.1	0.05	0.28	0.49	0.09	0.5
6	C179982-0001	WS	0.05	0.05	0.05	0.12	0.12	0.02	0.7
7	C180295-0001	WS	0.22	0.05	0.05	0.52	0.74	0.02	0.85
8	C179982-0003	WS	0.53	0.05	0.12	1.5	2.15	0.02	1.15
9	C180343-0001	WS	1.3	0.05	0.06	1.7	3.06	0.02	4.45
10	C181639-0006	WS	2.3	0.05	0.05	0.05	2.3	0.02	11.85
11	C177206-0002	WS	0.3	0.05	0.05	3.3	3.6	0.02	19.65
12	C180341-0002	WS	0.05	6.6	0.05	0.05	6.6	0.02	24.2
13	C180341-0001	WS	0.05	7.7	0.05	0.05	7.7	0.02	25.75
14	C179357-0001	WS	5.9	0.05	0.05	31	36.9	0.02	29
15	C179088-0001	WS	150	1.6	2.4	150	304	6.8	41.85
16	C181639-0007	WS	17	0.05	0.05	0.05	17	0.02	99.35
17	C181378-0001	WS	1.5	0.25	0.25	0.76	2.26	0.1	0.5
18	C181639-0005	WS	0.15	0.05	0.05	0.05	0.15	0.02	1.0
19	C180541-0001	WS	0.05	0.05	0.05	0.72	0.72	0.02	1.05
20	C180253-0001	WS	0.14	0.15	0.05	0.21	0.5	0.1	1.1
21	C179269-0001	WS	0.49	0.83	0.83	0.83	0.49	0.42	1.2
Total 2010 WS samples = 21									

The value of only one sample in counter #17 is above WHO threshold (1.0µg/L) by LC-MS/MS and below by ELISA. But the values of two variants (-YR and -RR) by LC-MS/MS in this sample have identical results and also the MDL is 5-folds higher than normal MDL (0.05µg/L) which represents the high matrix interference. Hence, the reliability of the LC-MS/MS results is questionable in this case and this particular sample can be considered invalid and can be excluded from this data set.

Values in counter #18 to #21 are close to *SDWA* (1.5µg/L) threshold by ELISA but below by LC-MS/MS. Possibly the higher values of ELISA are due to its sensitivity towards multiple

variant of microcystins. These three samples can also be further tested by PPIA if available to assess any potential environmental threat.

Scattered chart (Fig.8) was plotted from the data set provided in Table 11 in order to better understand the correlation between ELISA and LC-MS/MS results. ELISA values were represented on in y-axis while LC-MS/MS were in x-axis. A trend line was drawn and also the R^2 was calculated. The results from the scattered chart suggest a significant correlation between ELISA and LC-MS/MS of surface water from 2012 as shown by linear regression (R^2=0.8489). Two values in counter #19 and #20 were outliers. Both values are higher than *SDWA* threshold but are in agreement by both methods.

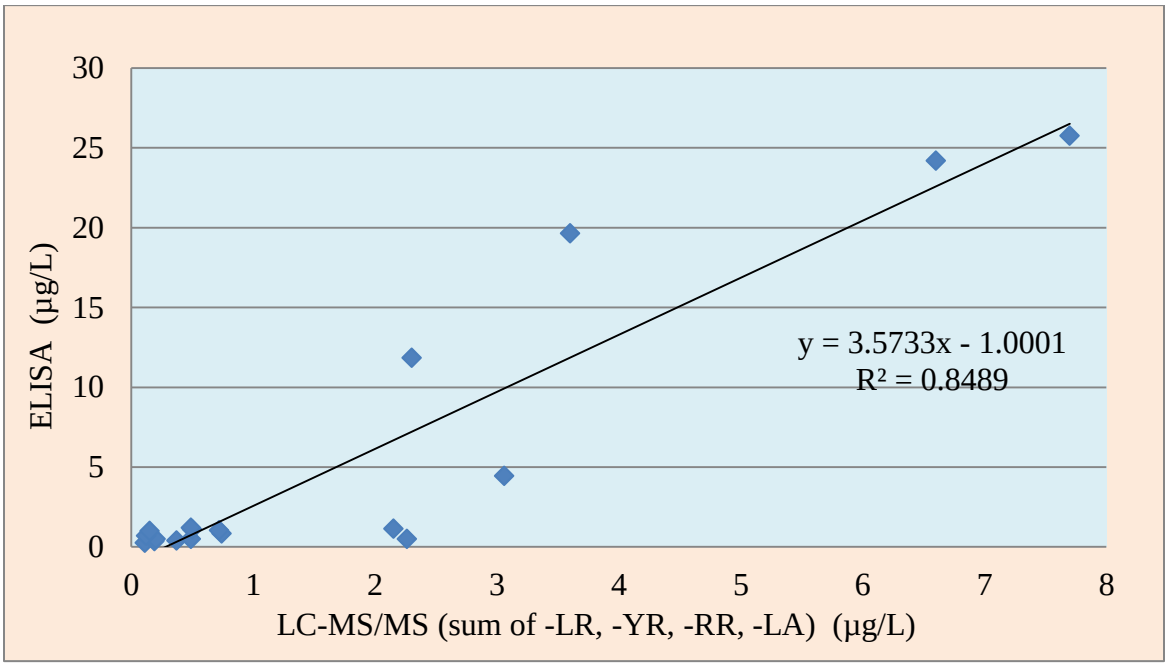

FIGURE 8. 2010 WS LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.2.1.4 Samples Negative by both ELISA and LC-MS/MS

Overall, there is a good agreement between ELISA and LC-MS/MS negative results represented in Appendix A-Table A1. However, in counter #2 to # 5, #19 and #20 MDL of LC-MS/MS had been elevated due to the matrix interference. In the case of counter # 5 the MDL was raised up to 2.5µg/L which is higher than the Ontario Safe Drinking Water Guidelines (1.5µg/L). This is an example when the usefulness of LC-MS/MS to measure health impact is hampered by matrix

interference. Such matrix interference is entirely absent in ELISA judging by the results in Table A1 (Appendix A).

3.2.2 2010 WS Agreement Calculation

The agreement between ELISA and LC-MS/MS can be divided in two groups. The first group includes all samples with an agreement between ESLIA and LC-MS/MS. The agreement (Table 11+Table A1) calculated for surface water samples in 2010 is 77% which comprised of 59/77 samples as shown in Table 12.

TABLE 12. 2010 SURFACE WATER (WS) AGREEMENT CALCULATION

2010 WS Test Results	**LC-MS/MS Tested**			**Total**
		Positive	**Negative**	
ELISA Tested	**Positive**	21	5	26
	Negative	13	38	51
		34	43	77
Agreement = 77%				

Five samples (counter #17 to #21) in Table 11 from the agreement group were found to have some discrepancy. In four samples (counter #18 to #21) out of these five, ELISA exhibits values equal or above WHO (1.0µg/L) guidelines and LC-MS/MS values well below. Perhaps the higher value of ELISA is due to it is cross reactivity with more variants of microcystins. These four samples can be further analyzed by PPIA to find out any potential toxicity. The fifth sample in counter #17 (Table 11) has the opposite scenario where cumulative value of all variants by LC-MS/MS is 2.25µg/L and ELISA value for the same sample is only 0.5µg/L. The MDL of LC-MS/MS in this case is very high and values of two variants are also identical. Probably the LC-MS/MS values needed to be adjusted due to high matrix interference. The results from this sample are not reliable.

The second group comprised of surface water samples from 2010 with an agreement between ESLIA and LC-MS/MS. The disagreement of 23% (Table 9+Table 10) consists of eighteen samples. Thirteen samples have values below the Ontario Drinking Water guidelines thus pose no threat to health and environment. However, rest of the five samples can be toxic as four (counter #8, #9, #12, #13) out of these five samples in Table 10 have values higher than *SDWA*

threshold (1.5µg/L) by LC-MS/MS but they are negative by ELISA. Perhaps the matrix interference or low specificity of antibodies from Abraxis kit towards the four variants (-LR, -YR, -RR, -LA) of microcystins. In the case of counter # 9 (Table 10) the identical values of two variants by LC-MS/MS show matrix interference. Hence the results from this counter are questionable.

The fifth sample (counter #5) in Table 9 has ELISA value 2.8µg/L and is negative by LC-MS/MS. The identical values of all four variants by LC-MS/MS and high MDL (2.0 µg/L) in this sample represents high matrix interference. Therefore the test result of this sample is unreliable and can be excluded from disagreement group.

3.2.3 2010 Drinking Water (WD) Results – Agreement between ELISA and LC-MS/MS

In 2010, there were 148 samples drinking water sample requested for ELISA and LC-MS/MS. These samples are divided into four Tables (13 to 15 and A2) according to the agreement between ELISA and LC-MS/MS results.

3.2.3.1 ELISA Positive and LC-MS/MS Negative Samples

Table 13 represents seven samples that are positive by ELISA and negative by LC-MS-MS. All these seven samples (counter #1 to #7) are below the Ontario Drinking Water Guidelines (1.5µg/L) therefore pose no health threat.

However, the value in counter #7 is above the WHO threshold of 1.0µg/L by ELISA and negative by LC-MS/MS. This sample if available can be further analysed by PPIA for the toxicity to avoid any harmful health impact. Sample in counter # 7 represents a potential threat that escaped LC-MS/MS detection but positive by ELISA.

TABLE 13. 2010 DRINKING WATER SAMPLES (WD) THAT WERE ELISA POSITIVE AND LC-MS/MS NEGATIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C177627-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
2	C179143-0001	WD	0.05	0.05	0.05	0.05	0.02	0.3
3	C180126-0002	WD	0.05	0.05	0.05	0.05	0.02	0.3
4	C178664-0001	WD	0.05	0.05	0.05	0.05	0.02	0.35
5	C180261-0001	WD	0.05	0.05	0.05	0.05	0.02	0.35
6	C178271-0001	WD	0.05	0.05	0.05	0.05	0.02	0.4
7	C178063-0001	WD	0.05	0.05	0.05	0.05	0.02	1.15
Total 2010 WS samples = 7								

3.2.3.2 ELISA Negative and LC-MS/MS Positive Samples

Table 14 categorize eleven samples that are negative by ELISA and positive by LC-MS/MS. Two common elements can be observed in the Table. First, all the ELISA values (Counter #1 to #8) are not truly non-detect. Instead they have a value of 0.15µg/L which is the MDL of ELISA.

TABLE 14. 2010 DRINKING WATER SAMPLES (WD) THAT WERE ELISA NEGATIVE AND LC-MS/MS POSITIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C177986-0001	WD	0.05	0.05	0.05	0.08	0.08	0.02	0.15
2	C177441-0001	WD	0.06	0.05	0.05	0.11	0.17	0.02	0.15
3	C178627-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.15
4	C178820-0001	WD	0.08	0.05	0.05	0.05	0.08	0.02	0.15
5	C179907-0001	WD	0.08	0.09	0.05	0.11	0.28	0.02	0.15
6	C179429-0001	WD	0.1	0.05	0.05	0.05	0.1	0.03	0.15
7	C179858-0001	WD	0.1	0.22	0.05	0.05	0.32	0.02	0.15
8	C179834-0001	WD	0.14	0.05	0.05	0.05	0.14	0.02	0.15
9	C179144-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	Not Detected
10	C180262-0001	WD	0.06	0.06	0.05	0.05	0.12	0.02	Not Detected
11	C179337-0001	WD	0.24	0.1	0.05	0.05	0.34	0.02	Not Detected
Total 2010 WD samples = 11									

The value of 0.15µg/L means that the detected values of ELISA lies between 0.05µg/L to 0.15µg/L and all the values within this range were rounded up to the significant number of 0.15µg/L. Counter #9, #10, #11 represent truly non-detect by ELISA when the signal were below 0.05µg/L.

Second the cumulative values of four variants (-LR,-YR, -RR, -LA) by LC-MS/MS (assuming 0.05µg/L is equal to zero µg/L) in all samples (counter#1 to #8) are slightly higher than ELISA but they are still well below the *SDWA* threshold (1.5µg/L).

In summary, all 11 samples in Table 14 pose no health threat even when ELISA was negative.

3.2.3.3 Samples Positive by both ELISA and LC-MS/MS

Table 15 illustrates a good agreement between ELISA and LC-MS/MS from Counter #1 to counter #25. However, in all these twenty five samples ELISA shows quite higher values than LC-MS/MS. Perhaps antibodies from Abraxis kit are able to react with multiple variants of microcystins therefore ELISA is producing higher results than LC-MS/MS.

In the cases of counter #23 to #25 the values by ELISA are close or higher than Ontario Drinking Water Guidelines (1.5µg/L) but this concern is not reflected by the low values of LC-MS/MS. All samples in this Table if available will be further investigated by PPIA to exclude any possible toxicity threat.

TABLE 15. 2010 DRINKING WATER SAMPLES (WD) THAT WERE POSITIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C178500-0001	WD	0.08	0.06	0.05	0.06	0.2	0.02	0.2
2	C179261-0001	WD	0.05	0.06	0.05	0.05	0.06	0.02	0.2
3	C179636-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.2
4	C179567-0001	WD	0.08	0.07	0.05	0.13	0.28	0.02	0.25
5	C180276-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.25
6	C180424-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.25
7	C178889-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.3
8	C179386-0001	WD	0.07	0.06	0.05	0.07	0.2	0.02	0.3
9	C178182-0001	WD	0.1	0.05	0.05	0.11	0.21	0.02	0.35
10	C178626-0001	WD	0.13	0.08	0.05	0.06	0.27	0.02	0.35
11	C179525-0001	WD	0.14	0.05	0.05	0.13	0.27	0.02	0.35
12	C180041-0001	WD	0.06	0.09	0.05	0.06	0.21	0.02	0.35
13	C180357-0001	WD	0.09	0.05	0.05	0.05	0.09	0.02	0.35
14	C178423-0001	WD	0.07	0.06	0.05	0.05	0.13	0.02	0.4
15	C179339-0001	WD	0.08	0.08	0.05	0.06	0.22	0.02	0.4
16	C177789-0001	WD	0.05	0.05	0.05	0.09	0.09	0.02	0.45
17	C180071-0001	WD	0.08	0.05	0.05	0.1	0.18	0.02	0.45
18	C179716-0001	WD	0.08	0.18	0.05	0.05	0.26	0.02	0.5
19	C178424-0001	WD	0.12	0.05	0.05	0.07	0.19	0.02	0.55
20	C179524-0001	WD	0.09	0.05	0.05	0.06	0.15	0.02	0.7
21	C178270-0001	WD	0.14	0.05	0.05	0.16	0.3	0.02	0.9
22	C179859-0001	WD	0.28	0.75	0.05	0.05	1.03	0.02	1.1
23	C179956-0001	WD	0.13	0.05	0.05	0.05	0.13	0.02	1.0
24	C180114-0001	WD	0.17	0.05	0.05	0.05	0.17	0.02	1.5
25	C179740-0001	WD	0.19	0.66	0.05	0.05	0.85	0.02	2.3
Total 2010 WD samples = 25									

Insignificant correlation (R^2=0.4342) was observed when a scattered chart (Fig.9) was plotted between ELISA and LC-MS/MS results (sum of variants by LC-MS/MS) from the data given in the Table 14.

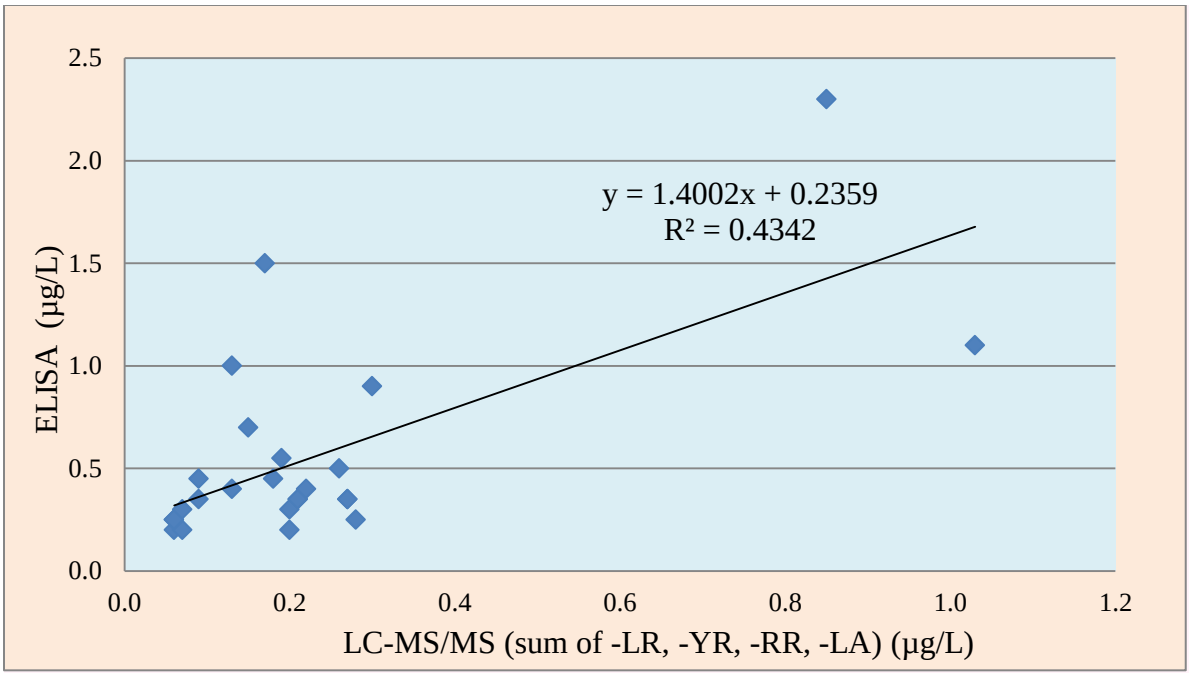

FIGURE 9. 2010 WD LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.2.3.4 Samples Negative by both ELISA and LC-MS/MS

Table A2 (Appendix A) represents unremarkable agreement between ELISA and LC-MS/MS values. .

3.2.4 2010 WD Agreement Calculation

Table 16 summarize all 148 drinking water samples tested in 2010. The agreement of 88% (Table 15+Table A2) between ELISA and LC-MS/MS comprised of 130 samples (25+105). Among these 130 samples, only three samples (counter #23 to #25 in Table 15) exhibit noticeable difference between values by ELISA and LC-MS/MS. ELISA values in these 3 cases were close to or above the WHO threshold (1.0µg/L) whereas LC-MS/MS showed low, non-threatening values.

TABLE 16. 2010 DRINKING WATER (WD) AGREEMENT CALCULATION

2010 WD Test Results	**LC-MS/MS Tested**			**Total**
		Positive	**Negative**	
ELISA Tested	**Positive**	25	7	32
	Negative	11	105	116
		36	112	148
Agreement = 88%				

The disagreement of 12% (Table 13+Table 14) consists of eighteen samples (7+11). The values of microcystins in the seventeen samples are below the threshold (1.5µg/L) of *SDWA*. Hence, they are considered to be safe drinking water in Ontario. Only one sample (counter #7 in Table 13) from this group had high values by ELISA and escaped detection by LC-MS/MS. The cross reactivity of antibodies with numerous variants of microcystins could explain why ELISA is a better detection method. All samples with higher ELISA values than LC-MS/MS or samples positive by ELISA and negative by LC-MS/MS will be considered for toxicity estimation by PPIA.

3.2.5 2010 WS and WD Agreement Calculation

Table 17 includes all drinking water and surface water samples from 2010. A good agreement of 84% between ELISA and LC-MS/MS supports the reliability of ELISA for microcystins analysis in water samples. Total water samples tested in 2010 were 225.

TABLE 17. 2010 SURFACE WATER (WS) AND DRINKING WATER (WD) AGREEMENT CALCULATION

2010 (WS &WD) Test Results	**LC-MS/MS Tested**			**Total**
		Positive	**Negative**	
ELISA Tested	**Positive**	46	12	58
	Negative	24	143	167
		70	155	225
2010 Agreement = 84%				

The agreement (Table 11+Table A1+Table 15+Table A2) part of 84% comprised of 189 samples. ELISA values in 7 samples (counter #18 to #21-Table 11, counter#23 to #25-Table 15) were found equal to or above the WHO threshold while LC-MS/MS values were not. Only one sample (counter # 17-Table 11) was above *SDWA* by LC-MS/MS but below *SDWA* by ELISA. However, the reliability of the LC-MS/MS results in this case is questionable because (1) its MDL is very high and (2) two variants exhibited identical values. These two observations occurred frequently in samples with high matrix interference rendering the LC-MS/MS results unreliable.

The 16% of disagreement (Table 9+Table 10+Table 13+Table 14) consist of 36 samples. Six samples from this group are in strong disagreement. Three samples (counter #8, #12, and #13 in

Table 10) out of these six were above *SDWA* threshold by LC-MS/MS and non-detect by ELISA. The value of fourth sample (counter # 9-Table 10) was also higher than *SDWA* threshold and non-detect by ELISA. However, in this case values of all four variants (-LR, -YR, -RR, -LA) have identical values by LCM-MS/MS, hence not a reliable test result. The fifth and sixth samples from disagreement group (counter #5-Table 9, counter #7-Table 13) had ELISA results higher than WHO limit (1.0µg/L) while LC-MS/MS missed it. ELISA missed only three samples out of 225 samples in 2010 where it was unable to detect microcystins in the water. These 3 surface water samples were toxic by *SDWA* and pose a health threat to wild life. Failure of ELISA to detect microcystins in these 3 cases remained unexplained. This phenomenon will be searched for in the following years to see if it occurs repeatedly.

3.3 2011 Agreement Calculation between ELISA and LC-MS/MS

Microcystins samples tested by both ELISA and LC-MS/MS in 2011 are sorted on matrix type in two groups; WS and WD. These two groups are discussed individually and then summarized collectively in one table for the agreement calculation of whole year (2011). The details of the agreement calculations are as follow

3.3.1 2011 Surface Water (WS) Results – Agreement between ELISA and LC-MS/MS

In 2011, the number of surface water samples tested for microcystins by ELISA and LC-MS/MS were 103. All samples are organized in Table 18 to 20 and Table A3 based on the agreement between ELISA and LC-MS/MS values.

3.3.1.1 ELISA Positive and LC-MS/MS Negative Samples

Table 18 represents the surface water samples that were positive by ELISA and negative by LC-MS/MS in 2011. Matrix interference is observed in counter #1, #4, #6 and #7 where values of all microcystins variants by LC-MS/MS are identical and MDL had been raised.

Values in counter #1 to #5 are below the *SDWA* threshold (1.5µg/L) by both methods therefore they pose no threat to the environment.

TABLE 18. 2011 SURFACE WATER SAMPLES (WS) THAT WERE ELISA POSITIVE AND LC-MS/MS NEGATIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C186289-0001	WS	0.83	0.83	0.83	0.83	0.33	0.2
2	C186356-0001	WS	0.05	0.05	0.05	0.05	0.02	0.2
3	C186120-0002	WS	0.05	0.05	0.05	0.05	0.02	0.35
4	C187404-0001	WS	1.2	1.2	1.2	1.2	0.5	0.4
5	C187829-0001	WS	0.05	0.05	0.05	0.05	0.02	0.55
6	C187406-0002	WS	0.5	0.5	0.5	0.5	0.2	2.3
7	C187565-0001	WS	2.5	2.5	2.5	2.5	1.0	32.7
Total 2011 WS samples = 7								

However, in all these counters (#1 to #5) ELISA exhibits slightly higher values than LC-MS/MS. ELISA values in counter #6 and #7 are above Ontario drinking water guidelines and below by LC-MS/MS. LC-MS/MS results of these two counters are unreliable because matrix interference caused the MDL in counter #6 and #7 to be raised 10- and 50-folds, respectively. PPIA will be performed on all these seven samples if available will be further investigated for toxicity analysis.

3.3.1.2 ELISA Negative and LC-MS/MS Positive Samples

Table 19 illustrates ELISA negative and LC-MS/MS positive surface water samples from 2011.

TABLE 19. 2011 SURFACE WATER SAMPLES (WS) THAT WERE ELISA NEGATIVE AND LC-MS/MS POSITIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C189074-0001	WS	0.35	0.5	0.5	0.5	0.35	0.2	0.15
2	C190314-0001	WS	0.19	0.05	0.05	0.05	0.19	0.02	0.15
3	C187701-0001	WS	0.05	0.05	0.07	0.05	0.07	0.02	Not Detected
4	C188786-0001	WS	0.05	0.05	0.05	0.14	0.14	0.02	Not Detected
5	C189233-0001	WS	0.05	0.05	1.5	0.05	1.5	0.02	Not Detected
6	C186289-0002	WS	0.44	0.25	0.25	0.25	0.44	0.1	Not Detected
7	C188329-0001	WS	0.05	0.05	0.05	0.05		0.42	Not Detected
8	C188890-0001	WS	0.19	0.5	0.5	0.13	0.32	0.2	Not Detected
Total 2011 WS samples = 8									

Values in counter #1 to #4 and counter #6 to #8 are well below the *SDWA* threshold (1.5µg/L) by both test methods. Hence they are considered no threat to wildlife or environment. Only one sample (counter #5) has value above the Ontario Drinking Water Guidelines by LC-MS/MS but non-detect by ELISA. This sample could potentially pose a borderline threat to wildlife and environment.

3.3.1.3 Samples Positive by both ELISA and LC-MS/MS

When samples in Table 20 are sorted by ELISA positivity in ascending order, they appear to fall into three groups. The first group comprises counter #1 to #22; exhibits a good agreement between ELISA and LC-MS/MS.

The second group is represented by three samples (counter #23, #24, #25) where the quantities of microcystins detected by ELISA are somewhat less than the sum of the LC-MS/MS variants. But both samples are still in agreement as they are positive by both methods. Counter #23 is unique; ELISA found 0.55µg/L of microcystins which were not detected by LC-MS/MS. Surprisingly, this sample contained 3000µg/L of Anatoxin-a. Matrix interference in this sample imposed a 10-fold elevation of MDL to 0.5µg/L.

The third group consists of counter #26 to #33 where ELISA results are 4 to 5-folds higher than LC-MS/MS. The trend in this group supports the hypothesis that ELISA can detect more variants of microcystins than LC-MS/MS. These samples if available will be further verified by using PPIA to assess the toxicity and potential health concerns.

TABLE 20. 2011 SURFACE WATER SAMPLES (WS) THAT WERE POSITIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C187658-0001	WS	0.1	0.05	0.06	0.09	0.25	0.02	0.4
2	C188624-0003	WS	0.11	0.05	0.05	0.14	0.25	0.02	0.45
3	C188326-0001	WS	0.05	0.05	0.11	0.05	0.11	0.02	0.5
4	C188624-0002	WS	0.1	0.05	0.05	0.05	0.1	0.02	0.5
5	C188624-0001	WS	0.1	0.05	0.05	0.05	0.1	0.02	0.65
6	C188252-0001	WS	0.29	0.05	0.05	0.06	0.35	0.02	0.75
7	C188064-0001	WS	1.1	0.5	0.5	0.96	2.06	0.2	4.5
8	C187406-0001	WS	0.8	0.5	0.5	0.7	1.5	0.2	6.4
9	C188326-0002	WS	2.4	2.5	3.5	2.5	5.9	1.0	13.05
10	C188395-0001	WS	0.85	0.05	0.05	4.0	4.85	0.02	20.05
11	C188947-0001	WS	8.1	0.55	0.57	0.49	9.71	0.28	35
12	C189234-0001	WS	2.5	2.5	5.7	2.5	5.7	1.0	50
13	C186621-0001	WS	12	0.12	0.12	12.0	12.0	0.05	63.9
14	C188145-0002	WS	39	1.2	0.61	1.5	42.31	0.07	67.97
15	C186812-0001	WS	39	16	4.3	14	73.3	0.2	81.6
16	C187436-0001	WS	6.6	3.4	1.0	1.0	12.0	0.5	95.6
17	C185928-0001	WS	28	5.0	5.0	5.1	33.1	2.0	126.5
18	C188252-0004	WS	30	14	2.4	2.4	48.8	0.02	170
19	C188252-0006	WS	56	3.2	8.6	1.8	69.6	0.45	240
20	C188252-0005	WS	4.5	2.7	0.74	0.18	8.12	0.02	270
21	C188252-0003	WS	90	5	200	28	323	0.24	1325
22	C189063-0001	WS	610	22	7.0	430	1069	0.1	3594.5
23	C188937-0001	WS	0.5	0.5	0.5	0.5	2.0	3000	0.55
24	C188145-0001	WS	67	2.0	1.4	1.8	72.2	0.02	50.4
25	C188889-0001	WS	340	5.0	5.0	2000	2340	2.0	700
26	C186120-0003	WS	0.13	0.25	0.25	0.24	0.37	0.1	1.5
27	C188252-0002	WS	0.18	0.05	0.37	0.12	0.67	0.02	1.5
28	C186241-0001	WS	0.06	0.08	0.05	0.05	0.14	0.02	1.55
29	C185986-0001	WS	0.08	0.05	0.05	0.29	0.37	0.02	2
30	C187700-0001	WS	0.05	0.05	0.64	0.08	0.08	0.02	2
31	C189075-0001	WS	0.27	0.05	0.05	0.07	0.34	0.02	2.05
32	C189213-0001	WS	0.35	0.5	0.5	0.5	0.35	2.2	2.65
33	C185988-0001	WS	0.26	0.05	0.33	0.4	0.66	0.13	2.85
Total 2011 WS samples = 33									

Scattered plot between ELISA and total LC-MS/MS was performed on the data in Table 20. Three samples (counter #21, #22, #25) found to be outliers. Although, the values of these outliers were in reasonable agreement between ELISA and LC-MS/MS buthese higher values diminished the correlation in the scatted plot. Hence, these three data points removed from the data set. A strong correlation was found by linear regression with a value of R^2=0.8875 (Fig.10).

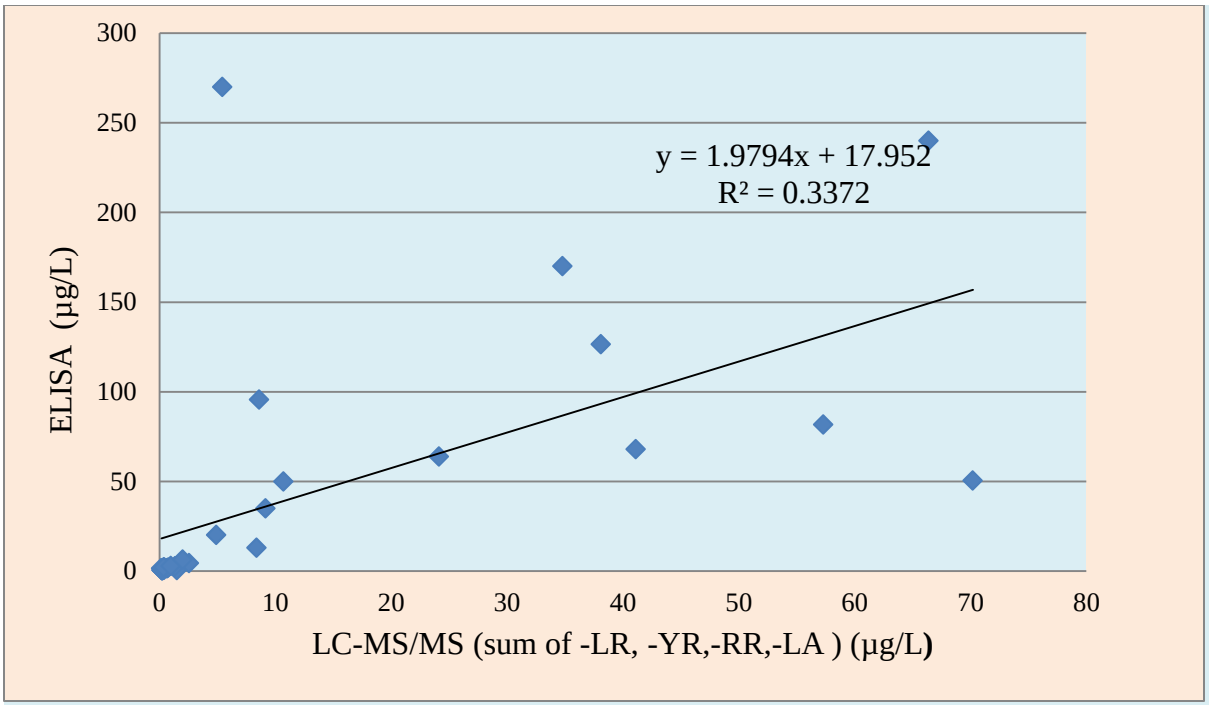

FIGURE 10. 2011 WS LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.3.1.4 Samples Negative by both ELISA and LC-MS/MS

Table A3 shows agreement between ELISA and LC-MS/MS negative test results for surface water in 2011. LC-MS/MS encountered matrix interference in 11out of 56 (20%) samples from Table A3, which is a considerable proportion.

3.3.2 2011 WS Agreement Calculation

The agreement calculated between ELISA and LC-MS/MS for surface water samples is 85% as shown in Table 21. This agreement (Table 20+Table A3) of 85% consists of 89 (33+56) samples. Eight samples (9%) from this group (counter #26 to #33 in Table 20) have ELISA values above *SDWA* threshold but below by LC-MS/MS.

TABLE 21. 2011 SURFACE WATER (WS) AGREEMENT CALCULATION

2011 WS Test Results		**LC-MS/MS Tested**		**Total**
		Positive	**Negative**	
ELISA Tested	**Positive**	33	7	40
	Negative	8	56	64
		41	63	104
2011 Agreement = 85%				

The disagreement (Table 18+Table 19) of 15% is composed of 15 (8+7) samples. Twelve samples had values below WHO by both methods. Thus they are safe for the environment. However, in 2 samples (counter #6, #7 in Table 18) values were above *SDWA* by ELISA and below by LC-MS/MS and in third sample (counter 5 in Table19) value of microcystins were equal to *SDWA* by LC-MS/MS and non-detect by ELISA. These three samples pose threat to wild animals and environment.

3.3.3 2011 Drinking Water (WD) Results – Agreement between ELISA and LC-MS/MS

Drinking water samples tested for microcystins in 2011 are organized in Table 22 to 24 and A4 according to the agreement between ELISA and LC-MS/MS test results.

3.3.3.1 ELISA Positive and LC-MS/MS Negative Samples

Table 22 summarize drinking water samples from year 2011, which were positive by ELISA and negative by LC-MS/MS.

Values in counter #1 to #20 are below the Ontario Drinking Water Guideline. Hence these samples pose no health threat. However, one value in counter #21 is above *SDWA* threshold by ELISA and below by LC-MS/MS. This sample will be analysed if available by PPIA to find out any potential toxicity by microcystins.

TABLE 22. 2011 DRINKING WATER SAMPLES (WD) THAT WERE ELISA POSITIVE AND LC-MS/MS NEGATIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C186476-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
2	C186607-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
3	C187240-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
4	C187241-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
5	C187285-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
6	C187467-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
7	C188170-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
8	C188344-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
9	C188586-0001	WD	0.05	0.05	0.05	0.12	0.02	0.2
10	C189306-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
11	C187946-0003	WD	0.05	0.05	0.05	0.05	0.02	0.25
12	C187667-0001	WD	0.05	0.05	0.05	0.05	0.02	0.3
13	C188000-0001	WD	0.05	0.05	0.05	0.05	0.02	0.3
14	C186475-0001	WD	0.05	0.05	0.05	0.05	0.02	0.35
15	C186479-0001	WD	0.05	0.05	0.05	0.05	0.02	0.35
16	C188906-0001	WD	0.05	0.05	0.05	0.05	0.02	0.35
17	C186478-0001	WD	0.05	0.05	0.05	0.05	0.02	0.4
18	C186531-0001	WD	0.05	0.05	0.05	0.05	0.02	0.4
19	C188544-0001	WD	0.05	0.05	0.05	0.05	0.02	0.45
20	C188633-0001	WD	0.05	0.05	0.05	0.05	0.02	0.7
21	C187841-0001	WD	0.05	0.05	0.05	0.05	0.02	7.85
Total 2011 WD samples = 21								

3.3.3.2 ELISA Negative and LC-MS/MS Positive Samples

Table 23 represents the drinking water samples found positive by LC-MS/MS and negative by ELISA in 2011.

Upon close examination of the Table 23, two observations can be made. First, all values by LC-MS/MS are below *SDWA* threshold (1.5µg/L) therefore posing no health threat. Second, ELISA values from counter #1 to #18 are not "non-detect" but rather the small quantities were rounded to the MDL of 0.15µg/L. By this rationale, 18 samples (counter #1 to #18) are in agreement and five samples (counter #19 to #23) truly disagree. Fortunately, all five LC-MS/MS values (counter #19 to counter #23) were much lower than Ontario Drinking Water Guidelines (1.5µg/L) by both methods. Therefore they pose no significant health threat either.

TABLE 23. 2011 DRINKING WATER SAMPLES (WD) THAT WERE ELISA NEGATIVE AND LC-MS/MS POSITIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C187466-0001	WD	0.07	0.07	0.05	0.05	0.14	0.02	0.15
2	C188210-0003	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.15
3	C187947-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.15
4	C188632-0001	WD	0.46	0.24	0.05	0.05	0.7	0.02	0.15
5	C188683-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.15
6	C188723-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.15
7	C188820-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.15
8	C188908-0001	WD	0.1	0.05	0.05	0.1	0.2	0.02	0.15
9	C189068-0001	WD	0.25	0.05	0.05	0.06	0.31	0.02	0.15
10	C189069-0001	WD	0.11	0.05	0.05	0.07	0.18	0.02	0.15
11	C189070-0001	WD	0.12	0.06	0.05	0.08	0.26	0.02	0.15
12	C189136-0001	WD	0.07	0.05	0.05	0.06	0.13	0.02	0.15
13	C189205-0001	WD	0.09	0.05	0.05	0.07	0.16	0.02	0.15
14	C189255-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.15
15	C189256-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.15
16	C189258-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.15
17	C189303-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.15
18	C189740-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.15
19	C186384-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	Not Detected
20	C187740-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	Not Detected
21	C188061-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	Not Detected
22	C188634-0001	WD	0.15	0.14	0.05	0.05	0.29	0.02	Not Detected
23	C189855-0001	WD	0.08	0.05	0.05	0.06	0.14	0.02	Not Detected
Total 2011 WD samples = 23									

3.3.3.3 ELISA and LC-MS/MS both Positive Samples

Table 24 consists of drinking water sample tested positive by both ELISA and LC-MS/MS in 2011. This Table can be divided into two groups. The first group includes the samples (counter #1 to #35) where values by both methods are in agreement. However, ELISA values are higher than LC- MS/MS.

TABLE 24. 2011 DRINKING WATER SAMPLES (WD) THAT WERE POSITIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C187887-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.2
2	C188435-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.2
3	C188467-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.2
4	C186879-0001	WD	0.08	0.07	0.05	0.05	0.15	0.02	0.25
5	C187284-0001	WD	0.06	0.09	0.05	0.05	0.15	0.02	0.25
6	C188436-0001	WD	0.07	0.06	0.05	0.05	0.13	0.02	0.25
7	C187665-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.35
8	C188824-0001	WD	0.12	0.05	0.05	0.05	0.12	0.02	0.35
9	C187496-0001	WD	0.06	0.06	0.05	0.05	0.12	0.02	0.4
10	C188130-0001	WD	0.09	0.09	0.05	0.05	0.18	0.02	0.4
11	C188511-0001	WD	0.08	0.05	0.05	0.05	0.08	0.02	0.4
12	C189743-0001	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.4
13	C189744-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.4
14	C186917-0001	WD	0.25	0.09	0.05	0.07	0.41	0.02	0.45
15	C188244-0001	WD	0.06	0.06	0.05	0.05	0.06	0.02	0.45
16	C186605-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.5
17	C187408-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.5
18	C187889-0001	WD	0.09	0.07	0.05	0.05	0.16	0.02	0.5
19	C188631-0001	WD	0.06	0.05	0.05	0.05	0.06	0.02	0.5
20	C188825-0001	WD	0.1	0.09	0.05	0.05	0.19	0.02	0.5
21	C186880-0001	WD	0.17	0.05	0.05	0.05	0.17	0.02	0.55
22	C186878-0001	WD	0.17	0.12	0.05	0.07	0.36	0.02	0.59
23	C186916-0001	WD	0.18	0.12	0.05	0.07	0.37	0.02	0.6
24	C188295-0002	WD	0.06	0.06	0.05	0.05	0.12	0.02	0.6
25	C187055-0001	WD	0.1	0.12	0.05	0.05	0.22	0.02	0.7
26	C188822-0001	WD	0.1	0.09	0.05	0.05	0.19	0.02	0.7
27	C187003-0001	WD	0.18	0.15	0.05	0.05	0.33	0.02	0.75
28	C187144-0001	WD	0.06	0.08	0.05	0.05	0.14	0.02	0.8
29	C187053-0001	WD	0.3	0.14	0.05	0.09	0.53	0.02	0.85
30	C188294-0003	WD	0.07	0.05	0.05	0.05	0.07	0.02	0.85
31	C188343-0001	WD	0.25	0.4	0.05	0.05	0.65	0.02	0.85
32	C186713-0001	WD	0.13	0.08	0.05	0.08	0.29	0.02	0.9
33	C189038-0001	WD	0.3	0.12	0.05	0.5	0.92	0.02	0.9
34	C186659-0001	WD	0.08	0.05	0.05	0.05	0.08	0.02	0.95
35	C189036-0001	WD	0.19	0.09	0.05	0.05	0.28	0.02	0.95
36	C188131-0001	WD	0.12	0.18	0.05	0.05	0.3	0.02	1.05
37	C187004-0001	WD	0.09	0.09	0.05	0.05	0.18	0.02	1.15
38	C187282-0001	WD	0.16	0.36	0.05	0.05	0.52	0.02	1.3
39	C188291-0003	WD	0.31	0.46	0.05	0.05	0.77	0.02	1.6
40	C188437-0001	WD	0.24	0.3	0.05	0.05	0.54	0.02	1.7
41	C188439-0001	WD	0.52	0.36	0.05	0.06	0.94	0.02	1.85
42	C187890-0001	WD	0.26	0.25	0.05	0.05	0.51	0.02	1.9

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
43	C188127-0001	WD	0.47	0.57	0.06	0.05	1.1	0.02	2.5
44	C187495-0001	WD	0.38	0.45	0.06	0.05	0.89	0.02	4.4
45	C187669-0001	WD	0.7	0.69	0.13	0.08	1.6	0.02	6.55
Total 2011 WD samples = 45									

The second group comprised of samples (counter #36 to #42 and #44) where ELISA values are close to or above the *SDWA* threshold (1.5µg/L) and below by LC-MS/MS. These eight samples will be investigated by PPIA if available for microcystins toxicity.

At a glance, a conclusion can be made from the values given in Table 24 that ELISA produce higher positive results of microcystins than LC-MS/MS, which was also observed in the data set from 2010. This reproducible observation supports our hypothesis that probably antibodies used in ELISA have the ability to cross react with multiple variants of microcystins, hence ELSIA produce higher positive results as compare to LC-MS/MS.

The scattered plot between ELISA and LC-MS/MS values in Table 24 represents an acceptable correlation (R^2=0.7024) ((Fig.11).

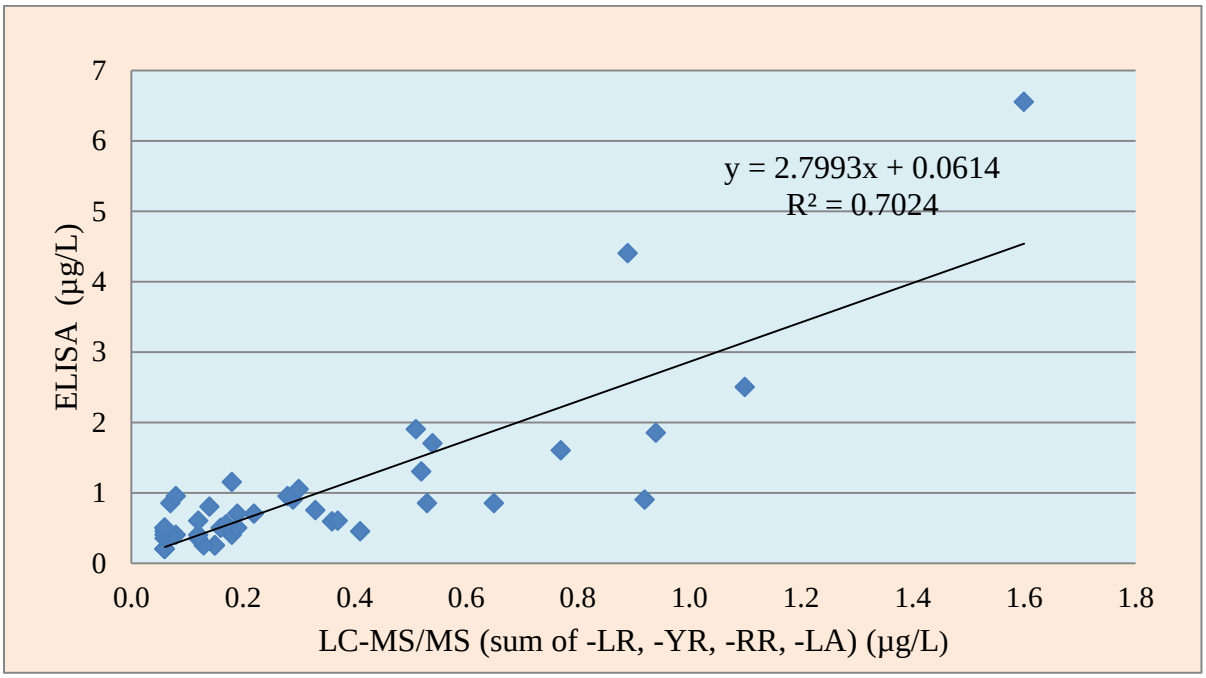

FIGURE 11. 2011 WD LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.3.3.4 Samples Negative by both ELISA and LC-MS/MS

Table A4 (Appendix A) lists the 83 drinking water samples that were negative by ELISA and LC-MS/MS. This group shows an unremarkable agreement between both methods

3.3.4 2011 WD Agreement Calculation

The number of drinking water samples tested by both ELISA and LC-MS/MS in 2011 was 172 and are summarized in Table 25.

TABLE 25. 2011 DRINKING WATER (WD) AGREEMENT CALCULATION

2011 WD Test Results	**LC-MS/MS Tested**			**Total**
		Positive	**Negative**	
ELISA Tested	**Positive**	45	21	66
	Negative	23	83	106
		68	104	172
2011 Agreement = 74%				

The agreement (Table 24+Table A4) calculated between both methods is 74% which is comprised of 128 (45+83) drinking water samples. Among these 128 samples, values of eight samples (counter #36 to #42, #45 in Table 24) were above WHO threshold by ELISA and below by LC-MS/MS.

The disagreement (Table 22+Table 23) of 26% consists of 44 (21+23) samples. Value of one sample (counter #21-Table 22) out of 44 was above *SDWA* threshold by ELISA and below by LC-MS/MS. In total, nine drinking water samples from 2011 posed potential health threats that deserve further toxicity assessment.

3.3.5 2011 - WS and WD Agreement Calculation

Table 26 summarized drinking and surface water samples from 2011 tested for microcystins according to the agreement between ELISA and LC-MS/MS values.

TABLE 26. 2011 (WS & WD) AGREEMENT CALCULATION

2011(WS &WD) Test Results		LC-MS/MS Tested		Total
		Positive	Negative	
ELISA Tested	Positive	78	28	106
	Negative	31	139	170
		109	167	276
Agreement = 79%				

The agreement (Table 20+Table A3+Table 24+Table A4) of 79% is composed of 217 (78+139) samples. Values of sixteen samples out of 216 were found above WHO threshold by ELISA and below by LC-MS/MS.

The disagreement (Table 18+Table 19+Table 22+Table 23) of 21% consist of (28+31) 59 samples. Three samples from this group have values above *SDWA* by ELISA and below by LC-MS/MS and one sample has opposite the scenario as the value of this sample is above *SDWA* by LC-MS/MS and non-detect by ELISA.

3.4 2012 Agreement Calculation between ELISA and LC-MS/MS

Microcystins samples tested by both ELISA and LC-MS/MS in 2012 are sorted on the base of matrix type into two groups; WS and WD. These two groups are discussed individually and then summarized collectively in one table for the agreement calculation of whole year (2012). The details of the agreement calculations are as follow:

3.4.1 2012 Surface Water (WS) Results – Agreement between ELISA and LC-MS/MS

The surface water samples tested in 2012 are organized in Table 27 to 29 and A5 according to the agreement between ELISA and LC-MS/MS values.

3.4.1.1 ELISA Positive and LC-MS/MS Negative Samples

Table 27 represent surface water samples tested positive by ELISA and negative by LC-MS/MS in 2012. This table can be divided into two categories.

TABLE 27. 2012 SURFACE WATER SAMPLES (WS) THAT WERE ELISA POSITIVE AND LC-MS/MS NEGATIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C196586-0004	WS	0.05	0.05	0.08	0.05	0.02	0.2
2	C196652-0004	WS	0.05	0.05	0.05	0.05	0.02	0.2
3	C195159-0002	WS	0.05	0.05	0.05	0.05	0.02	0.25
4	C196999-0003	WS	0.05	0.05	0.05	0.05	0.02	0.25
5	C195974-0001	WS	0.05	0.05	0.05	0.05	0.02	0.3
6	C195979-0003	WS	0.05	0.1	0.05	0.05	0.02	0.3
7	C196459-0001	WS	0.05	0.05	0.05	0.05	0.02	0.3
8	C196592-0002	WS	0.12	0.05	0.05	0.12	0.02	0.3
9	C196605-0003	WS	0.05	0.05	0.05	0.05	0.02	0.35
10	C198208-0001	WS	0.05	0.05	0.05	0.05	0.02	0.35
11	C196200-0003	WS	0.05	0.1	0.05	0.05	0.02	0.4
12	C196605-0002	WS	0.05	0.06	0.05	0.05	0.02	0.4
13	C196999-0001	WS	0.05	0.05	0.05	0.05	0.02	0.4
14	C196752-0001	WS	0.12	0.06	0.12	0.12	0.05	0.45
15	C196413-0003	WS	0.05	0.06	0.05	0.05	0.02	0.5
16	C197578-0001	WS	0.05	0.05	0.05	0.05	0.02	0.5
17	C197578-0002	WS	0.05	0.05	0.05	0.05	0.02	0.55
18	C197229-0002	WS	0.05	0.05	0.05	0.05	0.02	0.8
19	C197229-0001	WS	0.05	0.05	0.05	0.05	0.02	0.85
20	C197772-0012	WS	0.05	0.05	0.05	0.05	0.02	0.85
21	C195486-0001	WS	0.05	0.05	0.05	0.05	0.02	1.1
22	C196641-0001	WS	0.06	0.12	0.12	0.12	0.05	1.3
23	C196872-0001	WS	0.08	0.07	0.08	0.08	0.03	2.25
24	C196587-0001	WS	0.12	0.05	0.12	0.09	0.05	2.45
Total 2012WS samples = 24								

The first category includes the samples with values (counter#1 to #20) lower than the WHO threshold by both methods. Hence they pose no health or environmental threat. The positive ELISA values in these samples (counter #1 to #20) ranged from 0.2 to 0.85µg/L while LC-MS/MS were negative. Similar phenomena have been observed in previous data from 2010 and 2011.

The second category consists of four samples (counter #21 to #24) where values are above the WHO guideline (1.0µg/L) by ELISA and below by LC-MS/MS. In three samples (counter #22, #23, #24) two to three variants have identical values by LC-MS/MS. Also, the MDL had been raised to 1.6 to 2.4-folds in order to compensate for matrix interferences. Therefore, the test results in these three counters are not reliable. The fourth sample (counter #21) of this category is

negative by LC-MS/MS and value by ELISA is above the WHO threshold. Since the values of all four samples (#21 to #24) are above the WHO threshold, they could be toxic.

3.4.1.2 ELISA Negative and LC-MS/MS Positive Samples

Surface water samples that were tested negative by ELISA and positive by LC-MS/MS in 2012 are sorted in Table 28.

The values in the counter #1 to #15 are positive by LC-MS/MS and non-detect by ELISA or close to the MDL (0.15µg/L). This Table (28) is a good example to illustrate the limitations and failure of a sophisticated method like LC-MS/MS to produce useful results. The values of fifteen out of sixteen samples had been manipulated to overcome the effect of matrix interference. In doing so, identical results had been arbitrarily produced regardless of "true" variant concentrations which are not measureable in these cases. Hence LC-MS/MS cannot be relied upon as a method for precise results to detect microcystins in many cases of surface water. In contrast, ELISA technique is not affected by matrix interference and is therefore the method of choice for microcystins analysis.

TABLE 28. 2012 SURFACE WATER SAMPLES (WS) THAT WERE ELISA NEGATIVE AND LC-MS/MS POSITIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C196124-0001	WS	0.05	0.25	0.06	0.25	0.5	0.1	Not Detected
2	C193446-0001	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
3	C194522-0001	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
4	C196026-0002	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
5	C196149-0003	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
6	C196149-0004	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
7	C196752-0004	WS	0.25	0.25	0.25	0.25	1.0	0.1	Not Detected
8	C193850-0006	WS	0.5	0.5	0.5	0.5	2.0	0.2	Not Detected
9	C196458-0001	WS	0.5	0.5	0.5	0.5	2.0	0.2	Not Detected
10	C196026-0001	WS	1.3	1.3	1.3	1.3	5.2	0.5	Not Detected
11	C197894-0001	WS	5.0	5.0	5.0	5.0	20.0	2.0	Not Detected
12	C196124-0002	WS	0.25	0.25	0.21	0.25	0.96	0.1	0.15
13	C196130-0001	WS	0.25	0.25	0.25	0.25	1.0	0.1	0.15
14	C196532-0001	WS	0.25	0.25	0.25	0.25	1.0	0.1	0.15
15	C196997-0001	WS	0.25	0.25	0.25	0.25	1.0	0.1	0.15
16	C196196-0002	WS	0.05	0.05	0.05	0.05	0.2	0.21	0.15
Total 2012 WS samples = 16									

The sample in counter #16 is negative for microcystins by both methods. However, this sample is positive for antoxin-a (1.0µg/L).

3.4.1.3 Samples Positive by both ELISA and LC-MS/MS

The data in Table 29 was sorted into four groups. The first group includes the samples (counter #1 to #21) with an agreement between ELISA and LC-MS/MS. This group represent samples where ELISA values are always higher than LC-MS/MS. However, both methods produced the same toxicity evaluation with respect to the threshold values of drinking water guidelines.
The second group consists of ten samples (counter #22 to #31) with values above the WHO threshold (1.0µg/L) by ELISA and below by LC-MS/MS.

The first and second group (ELISA values higher than LC-MS/MS) will be analysed by PPIA for toxicity assessment.

The third group comprises of six samples (counter #32 to #37) where the values by both methods are in agreement. However, LC-MS/MS values are insignificantly (0.60- to 0.83-folds) higher than ELISA. The identical values for different variants of microcystins by LC-MS/MS in counter #33, # 34 and #37 represent high matrix interference, hence untrustworthy results.

The fourth group contains two samples (counter #38, #39) with values above the *SDWA* threshold (1.5µg/L) by LC-MS/MS and below by ELISA. These samples could be a potential threat to health or environment but identical values of different variants of microcystins by LC-MS/MS make the results of these counters unreliable.

TABLE 29. 2012 SURFACE WATER SAMPLES (WS) THAT WERE POSITIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C196548-0001	WS	0.15	0.05	0.09	0.05	0.15	0.02	0.2
2	C197394-0002	WS	0.24	0.05	0.05	0.18	0.42	0.02	0.65
3	C196759-0001	WS	0.15	0.05	0.05	0.06	0.15	0.02	0.75
4	C196897-0001	WS	0.22	0.08	0.08	0.08	0.22	0.03	0.85
5	C197218-0001	WS	0.37	0.05	0.05	0.19	0.56	0.02	0.9
6	C196587-0003	WS	0.25	0.25	0.25	0.39	1.14	0.1	2.85
7	C196027-0001	WS	0.52	0.12	0.44	0.52	1.48	0.05	2.95
8	C196275-0001	WS	0.5	0.5	0.5	0.5	2.0	0.03	3.5
9	C196493-0001	WS	0.34	0.09	0.85	0.05	1.19	0.02	3.7
10	C197376-0001	WS	0.05	2.7	0.05	0.05	2.7	7400	8.0
11	C196337-0001	WS	1.0	0.1	0.6	0.25	1.85	0.1	9.0
12	C197271-0001	WS	4.2	1.8	0.3	0.11	6.3	0.1	10.65
13	C196944-0001	WS	0.12	3.2	0.12	0.12	3.2	0.05	13.2
14	C196493-0002	WS	4.7	1.1	9.1	0.83	15.73	0.5	31.65
15	C195937-0001	WS	7.4	0.5	0.5	18	26.4	0.34	92.7
16	C196549-0001	WS	27	3.1	19	2.3	51.4	1.0	140.95
17	C197700-0001	WS	7.3	0.25	0.25	35	42.8	0.1	191.7
18	C196989-0001	WS	1.5	0.15	0.95	0.23	2.83	0.05	201.85
19	C196337-0002	WS	57	6.4	35	3.8	102.2	0.5	444.3
20	C196336-0001	WS	140	2.6	4.1	0.51	147.21	5.7	1122.05
21	C196114-0001	WS	330	36	140	64	570	1.0	2755.25
22	C196742-0001	WS	0.16	0.05	0.08	0.36	0.52	0.05	1.15
23	C196998-0001	WS	0.25	0.08	0.08	0.08	0.25	0.03	1.2
24	C196759-0002	WS	0.17	0.05	0.05	0.05	0.17	0.02	1.35
25	C197218-0002	WS	0.42	0.05	0.05	0.19	0.61	0.02	1.35
26	C197374-0001	WS	0.37	0.05	0.14	0.44	0.95	0.04	1.65
27	C197394-0001	WS	0.36	0.05	0.05	0.18	0.54	0.05	1.85
28	C196999-0002	WS	0.05	0.19	0.05	0.06	0.19	0.02	1.95
29	C196752-0002	WS	0.12	0.15	0.12	0.12	0.15	0.05	2.15
30	C197056-0001	WS	0.23	0.25	0.25	0.1	0.73	0.1	2.6
31	C196587-0002	WS	0.15	0.05	0.06	0.25	0.4	0.02	6.1
32	C196588-0001	WS	0.25	0.25	0.25	0.08	0.75	0.1	0.45
33	C196897-0002	WS	0.5	0.5	0.5	0.5	2.0	0.2	1.2
34	C197220-0001	WS	1.4	0.5	0.5	0.34	2.74	0.13	1.55
35	C196029-0001	WS	0.67	0.2	2.0	0.25	3.12	0.1	2.3
36	C196511-0001	WS	6.7	0.25	0.33	3.8	11.08	0.1	3.27
37	C194683-0001	WS	3.2	0.26	1.3	3.2	7.96	0.02	6.6
38	C196532-0002	WS	0.5	0.5	0.5	0.5	2.0	0.2	0.25
39	C196274-0001	WS	0.5	0.5	0.5	0.5	2.0	0.2	0.45
Total 2012 WS samples = 39									

The scattered plot between ELISA and LC-MS/MS positive values from Table 29 represents a strong correlation by linear regression with a value of R^2=0.9742 (Fig.12).

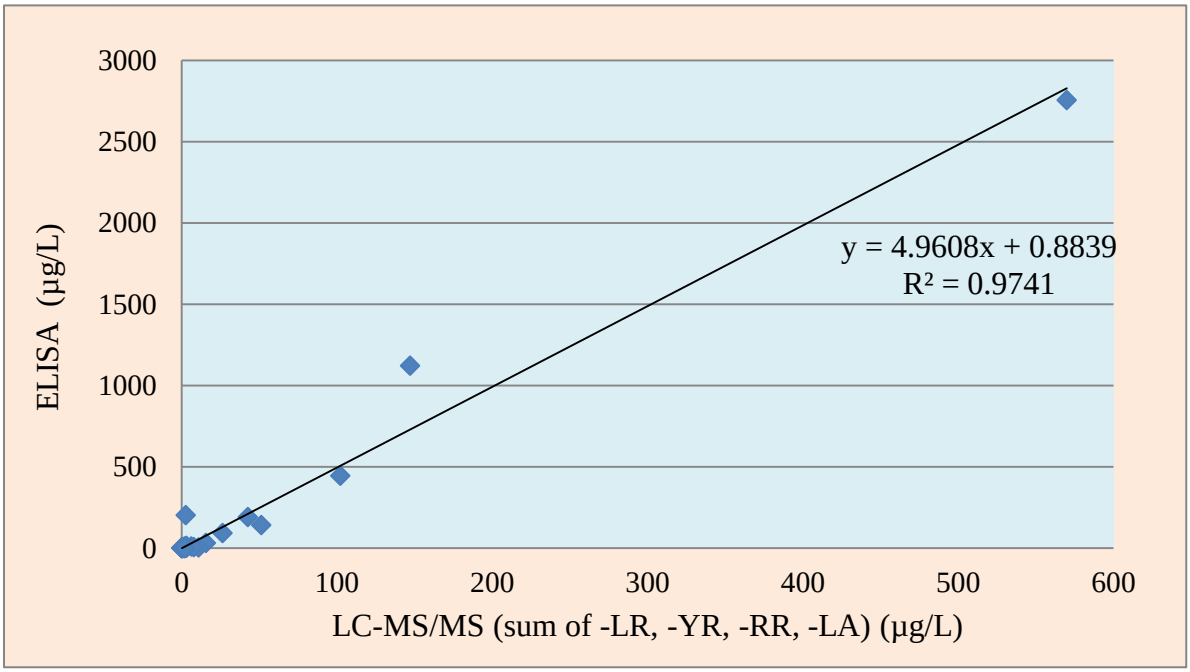

FIGURE 12. 2012 WS LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.4.1.4 Samples Negative by both ELISA and LC-MS/MS

Table A5 shows unremarkable agreement between ELISA and LC-MS/MS values. All 55 samples negative by ELISA are negative by LC-MS/MS too.

3.4.2 2012 WS Agreement Calculation

Table 30 summarizes the 134 surface water samples tested in 2012. The agreement (Table 29+Table 30) of 70% comprised of (55+39) 94 samples. Twelve samples among 94 have some discrepancy between ELISA and LC-MS/MS values. Ten samples (counter #22 to #31 in Table 29) have values above WHO threshold by ELISA and below by LC-MS/MS. Two samples are with values above WHO limit by LC-MS/MS and below by ELISA but at the same times these two samples shows unreliable LC-MS/MS results due to high matrix interference. Hence these two samples cannot be considered as LC-MS/MS positive samples.

TABLE 30. 2012 SURFACE WATER (WS) AGREEMENT CALCULATION

2012 WS Test Results		LC-MS/MS Tested		Total
		Positive	Negative	
ELISA Tested	Positive	39	16	55
	Negative	24	55	79
		63	71	134
Agreement = 70%				

The disagreement (Table 27+Table 28) of 30% includes (16+24) 40 samples. The values of twenty one samples are below the WHO threshold by both methods. Eighteen samples have unreliable results due to high matrix interference. There is one sample which is truly in disagreement with (Counter #21-Table 27) a value above WHO threshold by ELISA and below by LC-MS/MS.

3.4.3 2012 Drinking Water (WD) Results – Agreement between ELISA and LC-MS/MS

The drinking water samples tested in 2012 are organized in Table 34 to 52 according to the agreement between ELISA and LC-MS/MS values.

3.4.3.1 ELISA Positive and LC-MS/MS Negative Samples

Table 31 represents drinking water samples positive by ELISA and negative by LC-MS/MS form year 2012. ELISA values are higher than LC-MS/MS in counter #1 to #50 but are below WHO threshold by both methods, thus no potential health threat. Values in counter #51 to #61 are above WHO threshold by ELISA and below by LC-MS/MS. These samples could be toxic and if available, they will be investigated further for toxicity with PPIA.

TABLE 31. 2012 DRINKING WATER SAMPLES (WD) THAT WERE ELISA POSITIVE AND LC-MS/MS NEGATIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C196268-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
2	C196542-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
3	C196544-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
4	C198546-0001	WD	0.05	0.05	0.05	0.05	0.02	0.2
5	C195085-0001	WD	0.06	0.05	0.05	0.05	0.02	0.2
6	C196616-0001	WD	0.06	0.05	0.05	0.05	0.02	0.2
7	C197443-0001	WD	0.07	0.05	0.05	0.05	0.02	0.2
8	C199164-0001	WD	0.05	0.1	0.05	0.05	0.02	0.2
9	C197802-0001	WD	0.06	0.05	0.06	0.05	0.02	0.2
10	C195429-0001	WD	0.12	0.05	0.08	0.05	0.02	0.2
11	C197328-0001	WD	0.08	0.05	0.1	0.05	0.02	0.2
12	C196174-0001	WD	0.05	0.05	0.05	0.07	0.02	0.2
13	C195841-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
14	C196241-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
15	C196243-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
16	C196637-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
17	C196869-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
18	C197689-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
19	C198076-0001	WD	0.05	0.05	0.05	0.05	0.02	0.25
20	C196947-0001	WD	0.1	0.05	0.05	0.07	0.02	0.25
21	C197264-0001	WD	0.07	0.05	0.05	0.1	0.02	0.25
22	C197822-0001	WD	0.05	0.05	0.05	0.05	0.02	0.3
23	C198740-0001	WD	0.05	0.05	0.05	0.05	0.02	0.3
24	C197326-0001	WD	0.1	0.05	0.05	0.08	0.02	0.3
25	C196774-0001	WD	0.05	0.05	0.05	0.05	0.02	0.35
26	C196406-0001	WD	0.09	0.05	0.05	0.05	0.02	0.35
27	C195615-0001	WD	0.08	0.05	0.06	0.05	0.02	0.35
28	C197649-0001	WD	0.07	0.05	0.05	0.06	0.02	0.35
29	C196891-0001	WD	0.05	0.05	0.05	0.05	0.02	0.4
30	C196772-0001	WD	0.05	0.05	0.06	0.05	0.02	0.4
31	C195270-0001	WD	0.05	0.05	0.05	0.05	0.02	0.45
32	C198330-0001	WD	0.07	0.05	0.05	0.05	0.02	0.45
33	C195836-0001	WD	0.09	0.05	0.05	0.05	0.02	0.45
34	C197741-0001	WD	0.06	0.05	0.08	0.05	0.02	0.45
35	C196609-0001	WD	0.05	0.05	0.05	0.06	0.02	0.45
36	C196890-0001	WD	0.05	0.05	0.05	0.07	0.02	0.45
37	C198070-0001	WD	0.05	0.05	0.05	0.05	0.02	0.5
38	C196009-0001	WD	0.06	0.05	0.05	0.05	0.02	0.5
39	C196005-0001	WD	0.07	0.05	0.11	0.05	0.02	0.5
40	C197146-0001	WD	0.05	0.05	0.05	0.08	0.02	0.5
41	C196608-0001	WD	0.09	0.05	0.05	0.1	0.02	0.5
42	C198333-0001	WD	0.06	0.05	0.05	0.05	0.02	0.55

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
43	C196949-0001	WD	0.06	0.05	0.05	0.05	0.02	0.6
44	C196401-0001	WD	0.05	0.05	0.06	0.05	0.02	0.6
45	C195428-0001	WD	0.11	0.05	0.05	0.06	0.02	0.65
46	C195425-0001	WD	0.13	0.05	0.05	0.09	0.02	0.7
47	C196173-0001	WD	0.09	0.05	0.12	0.05	0.02	0.75
48	C196613-0001	WD	0.07	0.05	0.09	0.05	0.02	0.85
49	C197565-0001	WD	0.05	0.05	0.05	0.05	0.02	0.9
50	C198190-0001	WD	0.05	0.12	0.05	0.05	0.02	0.95
51	C195271-0001	WD	0.1	0.05	0.05	0.05	0.02	1.05
52	C197924-0001	WD	0.05	0.09	0.05	0.05	0.02	1.4
53	C197884-0001	WD	0.11	0.05	0.12	0.05	0.02	1.4
54	C197369-0001	WD	0.05	0.09	0.05	0.05	0.02	1.5
55	C197994-0001	WD	0.05	0.05	0.05	0.05	0.02	2.1
56	C198545-0001	WD	0.1	0.05	0.06	0.05	0.02	2.1
57	C196769-0001	WD	0.07	0.05	0.11	0.05	0.02	2.15
58	C196771-0001	WD	0.07	0.05	0.12	0.05	0.02	2.65
59	C196247-0001	WD	0.05	0.05	0.05	0.05	0.02	3.0
60	C196244-0001	WD	0.05	0.05	0.05	0.05	0.02	3.1
61	C199351-0001	WD	0.05	0.05	0.05	0.05	0.02	3.4
Total 2012 WD samples = 61								

3.4.3.2 ELISA Negative and LC-MS/MS Positive Samples

Table 32 represents drinking water samples that were tested negative by ELISA and positive by LC-MS/MS in 2012.

TABLE 32. DRINKING WATER SAMPLES (WD) THAT WERE ELISA NEGATIVE AND LC-MS/MS POSITIVE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C197019-0001	WD	0.05	0.15	0.05	0.05	0.15	0.02	0.15
2	C197478-0001	WD	0.1	0.05	0.09	0.05	0.13	0.02	0.15
3	C199020-0001	WD	0.05	0.24	0.05	0.05	0.24	0.02	0.15
4	C196889-0001	WD	0.07	0.05	0.13	0.05	0.13	0.02	0.15
5	C196246-0001	WD	0.13	0.05	0.15	0.05	0.28	0.02	0.15
6	C195468-0001	WD	0.18	0.05	0.1	0.09	0.18	0.02	Not Detected
Total 2012 WD samples = 6									

ELISA values in counter #1 to #5 are not truly non-detect; instead the low values are rounded up to 0.15µg/L which is the MDL of ELISA. The value of 0.15 means the ELISA value lies between 0.05µg/L to 0.15µg/L. By this rationale, there are no substantial differences between the values by both methods. The only sample with real disagreement is counter #6 where ELISA value is non-detect but it is positive by LC-MS/MS. However, the values of microcystins in counter #1 to #6 by both methods are below *SDWA* threshold (1.5µg/L). Hence these six samples pose no health threat.

3.4.3.3 Samples Positive by both ELISA and LC-MS/MS

Drinking water samples in Table 33 were tested positive by both ELISA and LC-MS/MS in 2012. Three groups are evident from table 36. The first group includes 19 samples (counter #1 to #19) where values by both methods are in good agreement. Second group consist of one sample (counter #20) with value equal to WHO threshold by LC-MS/MS and approximately so by ELISA. The difference in values between both methods is only 0.1µg/L which is negligible.

The third group consists of 19 samples (counter #21 to #39) with values equal or above WHO threshold by ELISA and below by LC-MS/MS. The samples from this group if available will be analysed by PPIA for toxicity.

TABLE 33. 2012 DRINKING WATER SAMPLES (WD) THAT WERE POSITIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA			
1	C196982-0001	WD	0.1	0.05	0.16	0.05	0.16	0.02	0.2
2	C197331-0001	WD	0.14	0.05	0.05	0.08	0.14	0.02	0.2
3	C199391-0001	WD	0.05	0.07	0.05	0.05	0.07	0.02	0.3
4	C195613-0001	WD	0.13	0.05	0.13	0.05	0.13	0.02	0.35
5	C197480-0001	WD	0.05	0.15	0.05	0.05	0.15	0.06	0.35
6	C197139-0001	WD	0.08	0.05	0.13	0.05	0.13	0.02	0.5
7	C197334-0001	WD	0.11	0.05	0.22	0.05	0.22	0.02	0.6
8	C198075-0001	WD	0.14	0.05	0.1	0.05	0.14	0.02	0.6
9	C197140-0001	WD	0.12	0.05	0.23	0.05	0.23	0.02	0.65
10	C197690-0001	WD	0.12	0.05	0.14	0.05	0.14	0.02	0.65
11	C195839-0001	WD	0.12	0.05	0.13	0.05	0.13	0.02	0.75
12	C197479-0001	WD	0.15	0.05	0.15	0.06	0.3	0.17	0.75
13	C197143-0001	WD	0.17	0.05	0.05	0.21	0.38	0.02	0.8
14	C197481-0001	WD	0.24	0.05	0.16	0.05	0.4	0.02	0.8
15	C196948-0001	WD	0.22	0.05	0.14	0.05	0.32	0.02	0.9
16	C195081-0001	WD	0.18	0.05	0.05	0.14	0.32	0.02	0.95
17	C196981-0001	WD	0.5	0.08	0.53	0.06	1.03	0.02	1.9
18	C197145-0001	WD	0.46	0.07	0.55	0.05	1.01	0.02	3.0
19	C196614-0001	WD	0.59	0.1	0.8	0.05	1.39	0.02	5.85
20	C197333-0001	WD	0.57	0.07	0.48	0.05	1.05	0.02	0.95
21	C196407-0001	WD	0.2	0.05	0.18	0.05	0.38	0.02	1.0
22	C195619-0001	WD	0.13	0.05	0.12	0.05	0.13	0.02	1.05
23	C197691-0001	WD	0.14	0.05	0.13	0.06	0.27	0.02	1.05
24	C197482-0001	WD	0.32	0.06	0.27	0.06	0.59	0.02	1.15
25	C195269-0001	WD	0.23	0.05	0.07	0.19	0.42	0.02	1.25
26	C197821-0001	WD	0.19	0.05	0.21	0.05	0.4	0.02	1.3
27	C196008-0001	WD	0.17	0.05	0.17	0.05	0.34	0.02	1.4
28	C196405-0001	WD	0.45	0.07	0.4	0.05	0.85	0.02	1.4
29	C197185-0001	WD	0.05	0.28	0.05	0.05	0.28	0.02	1.8
30	C197141-0001	WD	0.36	0.05	0.21	0.05	0.57	0.02	1.95
31	C197332-0001	WD	0.32	0.05	0.37	0.05	0.69	0.02	2.1
32	C196615-0001	WD	0.1	0.05	0.2	0.05	0.2	0.02	2.15
33	C196003-0001	WD	0.22	0.05	0.35	0.05	0.57	0.02	2.2
34	C195618-0001	WD	0.36	0.05	0.23	0.13	0.72	0.02	2.45
35	C198804-0001	WD	0.05	0.29	0.05	0.05	0.29	0.02	2.5
36	C196775-0001	WD	0.22	0.05	0.19	0.05	0.41	0.02	3.2
37	C198389-0001	WD	0.05	0.16	0.05	0.05	0.16	0.02	3.3
38	C198626-0001	WD	0.05	0.27	0.05	0.05	0.27	0.02	3.75
39	C195210-0001	WD	0.26	0.05	0.14	0.12	0.4	0.02	4.9
Total 2012WD samples = 39									

The scattered plot between ELISA and LC-MS/MS values from the Table 33 shows insignificant correlation (R^2=0.2216) (Fig.13).

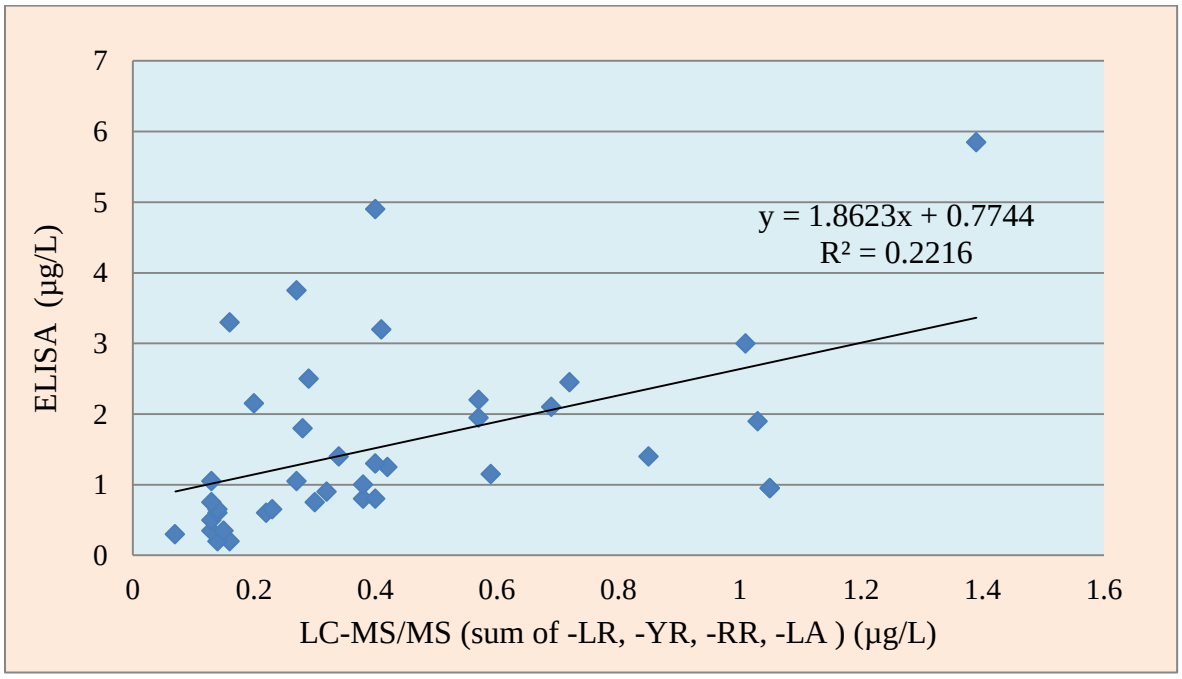

FIGURE 13. 2012 WD LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.4.3.4 Samples Negative by both ELISA and LC-MS/MS

Table A6 (Appendix A) shows a very good agreement between ELISA and LC-MS/MS values.

3.4.4 2012 WD Agreement Calculation

Table 34 represents drinking water samples (219) tested by both ELISA and LC- MS/MS in 2012. The agreement (Table 33+Table A6) of 69% consists of 152 (39+113) samples. Nineteen samples (counter #21 to 39) out of the 152 or 13% have values above WHO threshold by ELISA and below by LC-MS/MS.

Disagreement (Table 31+Table 32) of 31% consists of 67 (61+6) samples. Values of 56 samples are below the WHO threshold by both methods. The remaining 11 samples (counter#51 to #61 in Table 31) had values above WHO threshold by ELISA and below by LC-MS/MS. In total, 19+11=30 samples were above the WHO threshold by ELISA and below by LC-MS/MS. This represents 30/219=14% of all samples exhibiting warning by ELISA.

TABLE 34. 2012 DRINKING WATER (WD) AGREEMENT CALCULATION

2012 WD Test Results		LC-MS/MS Tested		Total
		Positive	Negative	
ELISA Tested	Positive	39	61	100
	Negative	6	113	119
		45	174	219
Agreement = 69%				

3.4.5 2012 WS and WD Agreement Calculation

Microcystins were analysed in 353 water samples (Surface and Drinking water) in 2012 (Table 35). The number of samples found in agreement (Table 29+Table A5+Table 33+Table A6) between ELISA and LC-MS/MS were 246 (70%). The values of 31 samples out of 246 or 13% were found above WHO threshold by ELISA and below by LC-MS/MS.

TABLE 35. 2012 (WS & WD) AGREEMENT CALCULATION

2012 (WS &WD) Test Results		LC-MS/MS Tested		Total
		Positive	Negative	
ELISA Tested	Positive	78	77	155
	Negative	30	168	198
		108	245	353
Agreement = 70%				

The disagreement (Table 27+Table 28+Table 31+Table 32) is 30% (77+30=107). Twelve samples had ELISA value above WHO threshold and below by LC-MS/MS.

3.5 Summary (WS & WD) of Agreement between ELISA and LC-MS/MS from 2010 to 2012

The summary of agreement between ELISA and LC-MS/MS over the period of three years is presented in Table 36.

TABLE 36. TOTAL 2010 TO 2012 (WS & WD) AGREEMENT CALCULATION

2010 to 2012 Test Results		LC-MS/MS Tested		Total
		Positive	Negative	
ELISA Tested	Positive	202	117	319
	Negative	85	450	535
		287	567	854
2010 to 2012 Agreement = 76%				

Total 854 water samples tested by ELISA and LC-MS/MS from 2010 to 2012 at MOE. The agreement between ELISA and LC-MS/MS is 76% and composed of 652 (202+450) samples while the disagreement of 24% consists of 202 (85+117) samples.

The purpose of agreement calculation was to analyse the differences between ELISA and LC-MS/MS values. The agreement calculation is based on MDL (ELISA 0.15µg/L, LC-MS/MS 0.05µg/L) and not the WHO (1.0µg/L) and *SDWA* (1.5µg/L) guidelines. Although some samples from agreement group were positive by both methods (based on MDL), at the same time they were safe by one method and toxic (values above WHO or *SDWA* threshold) by the other. Within the disagreement group, some samples were safe or toxic (values higher or lower than WHO or *SDWA*) by both methods even they were in disagreement.

Values of 757 (89%) samples among 854 from agreement and disagreement groups collectively were below the threshold of WHO (1.0µg/L) and *SDWA* (1.5µg/L) by both methods. Hence these samples pose no threat to health and environment.

The rest of the 97 (11%) samples had discrepancy between ELISA and LC-MS/MS test results: 72 samples had values above WHO threshold by ELISA and below by LC-MS/MS and 23 samples had values below WHO threshold by ELISA and above by LMCS.

Out of these 72 (8%) samples, 13 (2%) will be discounted from our final discussion because the results by LC-MS/MS for theses samples are not reliable. The rest of the 59(6%) samples from this group can be toxic and will be considered for the PPIA for further investigation.

Regarding the aforementioned 23 (3%) samples, 19 (2%) will be disregarded due to their unreliable LC-MS/MS results. In total, 4 (0.47%) samples had values above *SDWA* threshold (1.0µg/L) by LC-MS/MS and below by ELISA. These 4 samples with reliable LC-MS/MS results can be a threat to wildlife and environment. The values of 2 samples were above the 0.02µg/L for antitoxins by LC-MS/MS and below by ELISA.

3.5.1 Summary of Regression Analyses from 2010 to 2012

Among 2010 to 2012 samples with results higher than the method detection limits (MDLs), the correlation between ELISA and LC-MS/MS was weak in surface water (R^2=0.422, p=0.0001, n=109) (Fig.14) and similarly week in drinking water (R^2=0.386, p=0.0001, n=93) (Fig. 15).

TABLE 37. SUMMARY OF REGRESSION ANALYSIS FROM 2010 TO 2012

	2010 WS	2010 WD	2011 WS	2011 WD	2012WS	2012WD
N	46	25	33	45	39	39
Slope	3.5733	1.4002	1.9794	2.7993	4.9608	1.8623
R^2	0.8489	0.4342	0.3372	0.7024	0.9741	0.2216
P	0.111	0.0003	0.0012	0.0001	0.0001	0.002

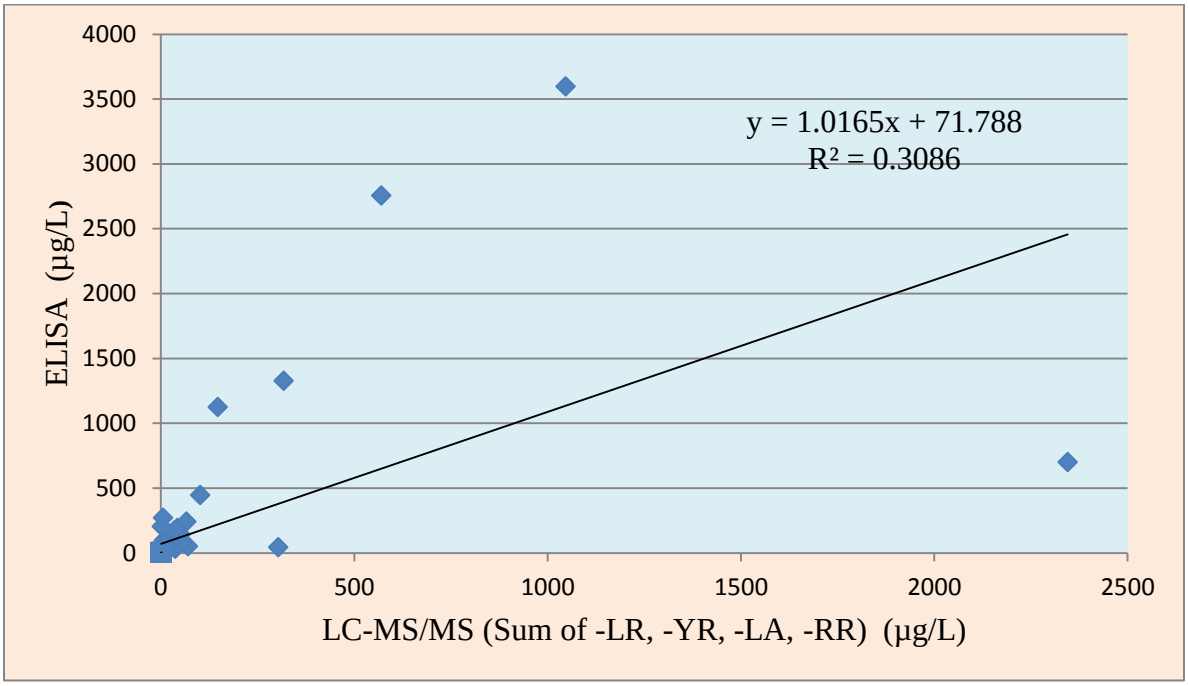

FIGURE 14. 2010-2012 WS LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

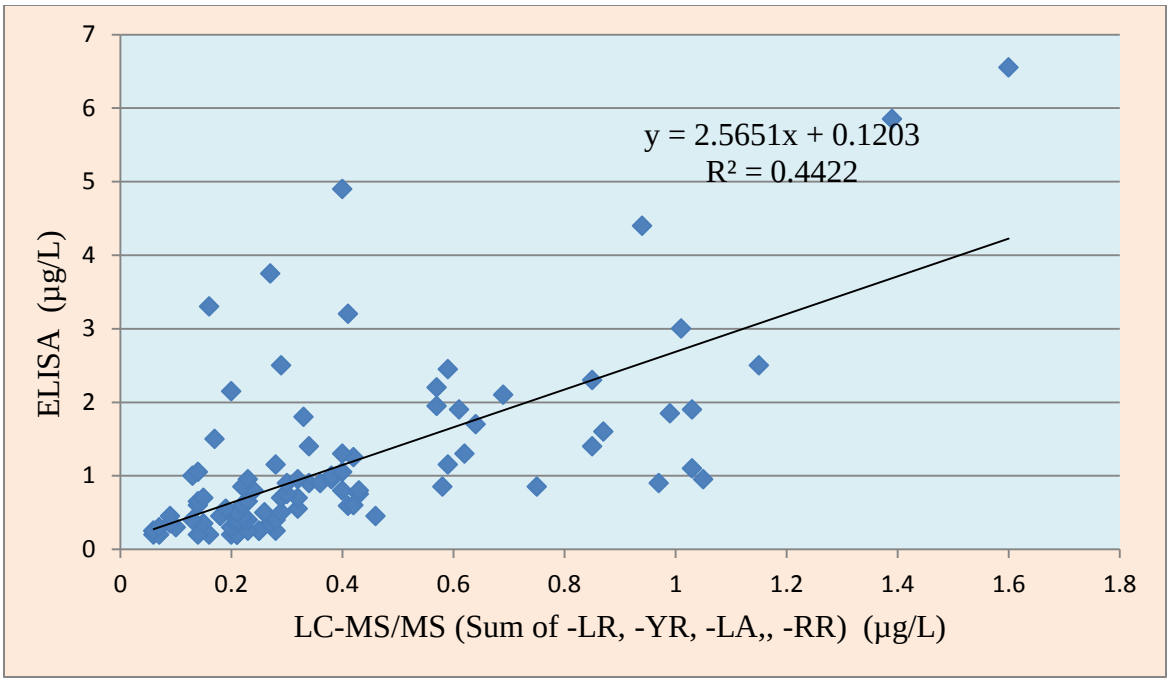

FIGURE 15. 2010-2012 WD LINEAR REGRESSION ANALYSIS BETWEEN ELISA AND LC-MS/MS POSITIVE RESULTS OF MICROCYSTINS

3.6 Discussion

Microcystins data from 2010 to 2012 indicates that 22% (186) of samples exhibited ELISA values higher than LC-MS/MS but still below WHO guidelines, while in 8% (59) of the cases, values of ELISA were above the WHO guidelines but below by LC-MS/MS. Thus, in a significant portion of 30% cases, ELISA values were higher than LC-MS/MS, which supports the hypothesis of this research that ELISA detects total microcystins.

Only 4 cases of LC-MS/MS positives out of 853, ELISA failed to detect microcystins; 3 from 2010 and 1 from 2011 but this pattern did not exist in 2012. Perhaps over a period of time, the manufacturer has improved the quality of their ELISA kit, or perhaps the ELISA operators have improved proficiency and performance. Perhaps both are true. In any case, the latest performance data in 2012 show that ELISA did not miss any true positive sample. The summary of discrepant results between ELISA and LC-MS/MS is available in Fig.16.

The analysis of microcystins data also reveals that 59 (41WD+18WS) (7%) results were above the WHO threshold (1µg/L) by ELISA but these samples were negative by LC-MS/MS. Clearly

relying on LC-MS/MS results can put public health, wild animals and environment in danger. To avoid the toxic exposure of microcystins, all water samples (WS or WD) should be analysed with ELISA first. These results are also a push towards the change of *O. Reg. 169/03* from only -LR to total microcystins.

Matrix interference has considerable impact on the LC-MS/MS results (Table C1 and C2-AppendixC). LC-MS/MS in 90 samples (11%) out of 854 (microcystins test results of samples from 2010 to 2012) have failed to produce reliable results. Matrix interference effect is more evident in surface water samples {75 (9%)} as compared to drinking water {15 samples (2%)}. LC-MS/MS cannot claim that any of these results are correct due to the lack of availability of matrix reference material. The long tedious 3 to 4 days of sample preparation could not help LC-MS/MS to produce the reliable results. On the contrary, ELISA produced consistent results without any matrix interference within a day.

Microcystins were being analysed by LC-MS/MS from 2002 to 2009 at MOE in surface and drinking water samples (raw and treated water). From 2010, after the introduction of ELISA technique, treated and raw water samples are being screened by ELISA and positive samples by ELISA are confirmed by LC-MS/MS while surface water are analysed by LC-MS/MS only. A summary of agreement calculation for each matrix type(WS & WD) and for each year is available in Fig.17

The data analysis from 2010 to 2012 demonstrates that when it comes to the surface water samples, LC-MS/MS cannot be relied upon as a technique of choice due to high effects of matrix interferences whereas ELISA results were reliable (Carmichael & An, 1999; Zeck *et al., 2001;* Metcalf *et al.,* 2001; Fischer *et al.,* 2001; Sheng *et al.,* 2006) Hence, it is suggested that ELISA should be used for the analysis of microcystins in all samples including drinking water and especially surface water samples because this method is the fit-for–purpose (method is suitable for the intended purpose) (Carmichael & An, 1999; Pírez, Macarena *et al.,* 2013; Sangolkar *et al.,* 2006). Perhaps, the samples should be only tested by LC-MS/MS when a concentration of certain variant of microcystins is required.

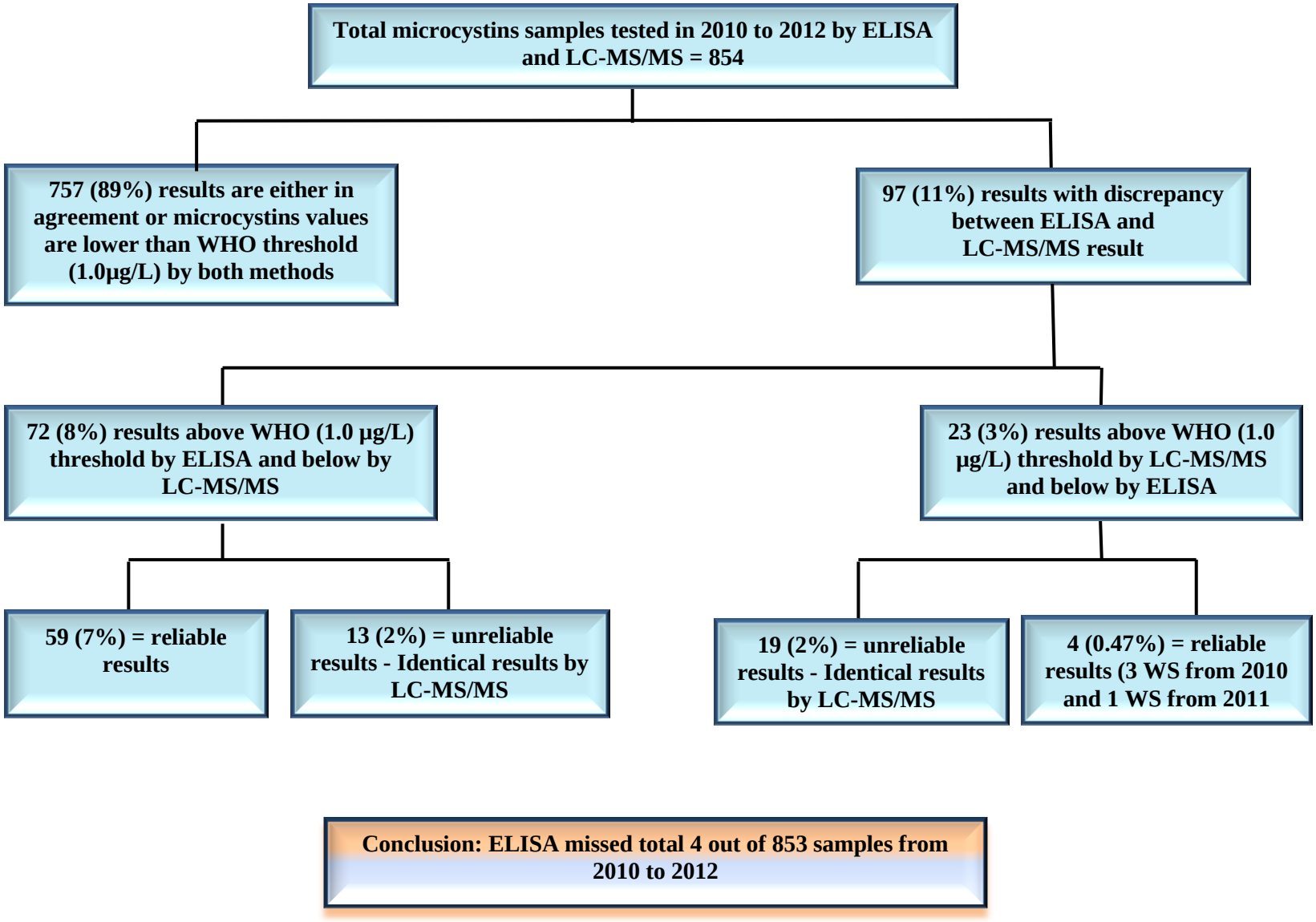

FIGURE 16. ANALYSIS OF 2010 TO 2012 MICROCYSTINS RESULTS OF ELISA AND LC-MS/MS

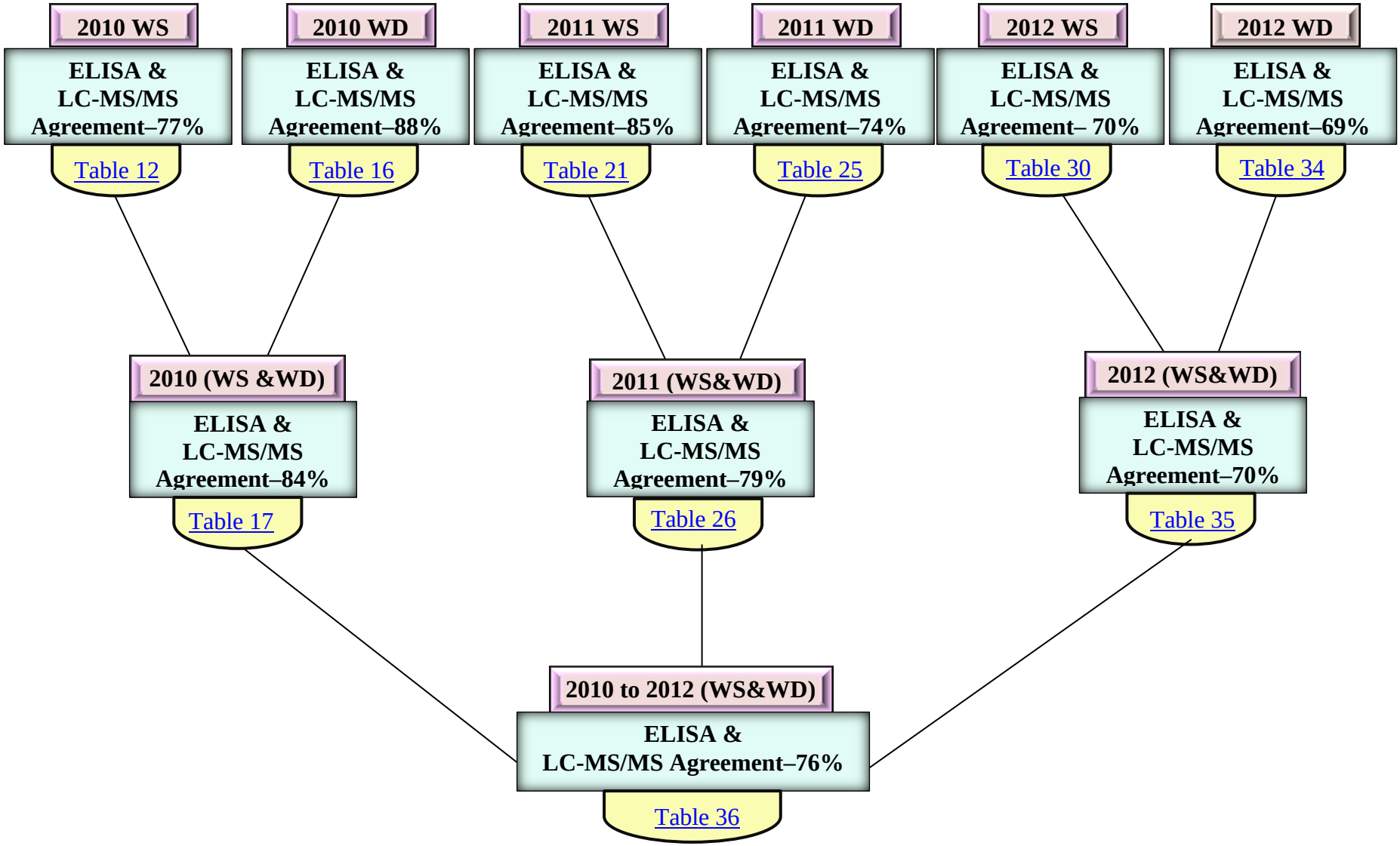

FIGURE 17. AGREEMENT CALCULATION BETWEEN ELISA AND LC-MS/MS FROM 2010 TO 2012

3.7 Anatoxin-a Positive Samples from 2010 to 2012

Anatoxin-a was positive in 19 samples from 2010 to 2012. However, 9 samples from counter #11 to counter # 19 will be ignored due to their unreliable results by LC-MS/MS.

TABLE 38. ANATOXIN-A POSITIVE DRINKING AND SURFACE WATER SAMPLES FROM 2010 TO 2012

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C180253-0002	WS	0.11	0.1	0.05	0.28	0.09	0.5
2	C197478-0001	WD	0.1	0.05	0.09	0.05	0.13	0.12
3	C188145-0002	WS	39	1.2	0.61	1.5	0.07	67.97
4	C180253-0001	WS	0.14	0.15	0.05	0.21	0.1	1.1
5	C188252-0003	WS	90	5	200	28	0.24	1325
6	C188947-0001	WS	8.1	0.55	0.57	0.49	0.28	35
7	C188252-0006	WS	56	3.2	8.6	1.8	0.45	240
8	C188329-0001	WS	0.05	0.05	0.05	0.05	0.42	Not Detected
9	C179589-0001	WS	0.05	4.1	0.05	0.29	0.46	Not Detected
10	C179089-0001	WS	27	0.33	0.38	14	2.0	Not Detected
11	C179406-0001	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
12	C196124-0001	WS	0.05	0.25	0.06	0.25	0.1	Not Detected
13	C193446-0001	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
14	C194522-0001	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
15	C196026-0002	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
16	C189213-0001	WS	0.35	0.5	0.5	0.5	2.2	2.65
17	C179088-0001	WS	150	1.6	2.4	150	6.8	41.85
18	C188937-0001	WS	0.5	0.5	0.5	0.5	3000	0.55
19	C197479-0001	WD	0.15	0.05	0.15	0.06	0.17	0.75

Table 38 represents all samples (WS and WD) tested positive by either ELISA or LC-MS/MS or by both. The values of anatoxin-a in 7 samples are (counter #1 to #7) in agreement for anatoxin-a between ELISA and LC-MS/MS.

Values in 3 counters #8 to #9 for anatoxin-a were non-detect by ELISA and positive by LC-MS/MS. However, values of anatoxin-a in counter #8 and #9 are well below the WHO threshold. Hence, they pose no health effect. Whereas, 1 sample (counter#9) is truly positive with the value of anatoxin-a is higher than *SDWA* and it is non-detect by ELISA. This sample poses a health threat.

All ELISA tests at Ministry of the Environment (MOE) are performed by using Abraxis kit. The antibodies in this kit are only specific against ADDA moiety of microcystins structure not against

anatoxin-a. Hence, if only ELISA (Abraxis kit) is used for the detection of anatoxin-a in the future, there are very good chances that ELISA can miss the anatoxin-a. But to solve this problem, Abraxis (manufacturer) recently (April 2013) developed an anatoxin-a kit based receptor binding (Van Apeldoorn *et al.*, 2007). The principle and method in detail is available online at http://goo.gl/Bu1t7A.

4 Cost Comparison and Workload Contribution between ELISA and LC-MS/MS

ELISA and LC-MS/MS results were collected from Laboratory Services Branch of MOE. Workload division and cost comparison calculation between ELISA and LC-MS/MS was performed from 2010 to 2012. This chapter will analyse the workload and the cost effectiveness of ELISA versus LC-MS/MS for the analysis of microcystins at MOE. The details are as follow:

4.1 Material and Method

Data received from MOE data base for the analysis of microcystins from 2010 to 212 was sorted on the basis of detection method. Data was sorted and categorized into three groups; (1) samples tested by ELISA only, (2) samples tested by LC-MS/MS only and (3) samples tested by both. The details of sorted data are available in Appendix E (Table E1 to Table E18).

4.1.1 Workload Calculation

Data was further sorted on the basis of matrix type for each year. The workload calculations were performed in two different ways. Firstly, workload was calculated on the yearly basis (Table D1 to D9 in Appendix D) and also collectively compiled for three years (2010 to 2012) in Table D10. Secondly, the data was also sorted on the basis of individual matrix type (WS or WD) over the period of three years (2010 to 2012) (Table 42, Table 43). The workload calculation was performed by using the following table:

TABLE 39. WORKLOAD CALCULATION

Year and Matrix Type (WS or WD)	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	Samples tested by both **(a)**	Samples tested by only ELISA not by LC-MS/MS **(b)**	Total samples tested by ELISA
	No	Samples tested by only LC-MCS/SM not by ELISA **(c)**		Samples tested by LC-MS/MS only
		Total samples tested by LC-MS/MS **(d)**		Total samples tested by both methods **(e)**
Percentage of microcystins workload relieved by ELISA				**b/e%**
LC-MS/MS contribution to total workload				**d/e%**

4.1.2 Cost Comparison

Cost of LC-MS/MS and ELISA were estimated based on operations at the Laboratory Services Branch (LaSB) of MOE.

The LC-MS/MS method required multiple equipment for sample preparation and analysis e.g. lyophilizer, bottle rotators, centrifuge, membrane filtration apparatus and LC-MS/MS system. The estimated cost for LC-MS/MS equipment is $300,000 assuming a 10-years life time. Thus, the average cost is $30,000 per year. The reagents and maintenance of $10,000 per year was added to the equipment cost ($30,000+$10,000=$40,000). Microcystins analysis by LC-MS/MS is performed by four employees at LaSB lab including two senior scientists, 1 full time technician and one full time co-op student. However, two scientists also perform other analysis too. Hence, salaries of two scientists were assumed equal to one full time junior scientist salary. The total salaries are approximately $200,000 for one year. Therefore, the estimated cost for LC-MS/MS analysis is about $240,000 per year. An average cost of $365 per sample is calculated based on the cost per year divided by the average number of samples per year (657).

Estimated cost of ELISA equipment is $30,000 assuming a 10-years life span. Average cost per year would be $3,000. One kit suffices the analysis of 36 samples and the cost per kit is $320. ELISA cost to analyse 657 samples per year is 36x657=18kits=18x$320=$5,760. ELISA consumables such as pipette tips and plastic vials are approximately $500 per year; $5,760+$500=$6,260. Therefore, the material cost alone is $6,260/657=$9.50 per sample. Annual salary of one fulltime employee is approximately $80,000 to perform the ELISA. Thus, the total estimated cost per year is $6,260+$80,000=$89,260. An average cost of $136 per sample by ELISA is arrived at by dividing the total cost per year by the number of samples tested per year (657).

The cost calculations are rough approximations. These calculations exclude amortization, building, hydro and overhead. Cost of $300,000 for LC-MS/MS and $30,000 for ELISA were already budgeted as capital expenditure and was paid in cash to avoid any interest. The useful life of equipment was determined to be 10 years and straight line depreciation method was used to come up with the yearly cost. The details are tabulated in Table 40.

TABLE 40. COST CALCULATION OF ELISA AND LC-MS/MS FOR THE DETECTION OF MICROCYSTINS AT MOE

	LC-MS/MS Cost		ELISA Cost			
Instrument Cost	$300,000/10years	$30,000 per year	$30,000/10years			$3,000 per year
Reagents + Maintenance + Supplies Per year		$10,000	Each kit=$320, 36 samples/kit	656/36= 18 kits	18x320+$500 (Supplies)	$6,260
Labour Cost per year	2 Sr. Scientist equivalent to 1 FTE*, 1 Junior Scientist, 1 Tech, 1 Student	$200,000	1 FTE* (1 Full time Equivalent)			$80,000
Total cost per year		$240,000				$89,260
Cost Per Sample		**$365.67**				**$136.00**

* FTE - Full time Equivalent, Cost per sample is based on the cost per year by each method divided by the average number of sample tested per year at MOE (656 samples per year).

4.2 Results and Discussions

Ontario Regulation O. Reg. 248/03 was amended in 2010 to allow the use of ELISA for water (WD) testing (MOE, 2010a). Over a period of three years (2010 to 2012) total 1970 test results of surface water and drinking water were generated by a combination of ELISA and LC-MS/MS methods (Table 41).

TABLE 41. SUMMARY OF ELISA AND LC-MS/M WORKLOAD CONTRIBUTION FROM 2010 TO 2012 FOR THE DETECTION OF MICROCYSTINS (WS & WD)

	2010		2011		2012		Total
	WS	**WD**	**WS**	**WD**	**WS**	**WD**	
Samples Tested by ELISA Only	40	206	7	335	59	423	1070
Samples Tested by LC-MS/MS Only	0	13	0	10	11	12	46
Samples Tested by both ELISA and LC-MS/MS	77	148	104	172	134	219	854
Total samples tested in Each Year	117	367	111	517	204	654	1970
Percentage of Workload Relieved by ELISA	34%	56%	6%	68%	29%	65%	54%
Percentage of Workload Relieved by LC-MS/MS	66%	44%	94%	32%	71%	35%	46%

Details of the work load calculation for each matrix type(WS & WD) in each year year are available in Appendix D (Table D1, Table D2, Table D3, Table D4, Table D5, Table D6, Table D7, Table D8, Table D9, TableD10..

Among these 1970 samples, 1538 were drinking water (raw and treated) samples. ELISA shared 63% of the workload for drinking water samples from 2010 to 2012. Clearly, clients prefer ELISA over LC-MS/MS by a majority. This preference is illustrated in Table 42.

TABLE 42. DRINKING WATER WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS FROM 2010 TO 2012

Drinking Water Samples Tested in 2010 2012	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	539	964	1503
	No	35		35
		574		1538
Percentage of microcystins workload relieved by ELISA				**63%**
LC-MS/MS contribution to total workload				**37%**

As mentioned earlier, the regulation was amended only for the screening of drinking water. Most of raw water and surface water samples are still being analysed by LC-CMS/MS at MOE. Hence, ELISA contribution is not evident in the surface water samples as it is in drinking water samples. ELISA shared a workload of 25% (Table 43) of surface water samples and 63% (Table 42) of drinking water samples for the detection of microcystins from 2010 to 2012.

TABLE 43. SURFACE WATER WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS FROM 2010 TO 2012

Surface Water Samples Tested in 2010 to 2012	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	315	106	421
	No	11		11
		327		432
Percentage of microcystins workload relieved by ELISA				**25%**
LC-MS/MS contribution to total workload				**75%**

Collectively (WS and WD), ELISA shared a workload of 54% from 2010 to 2012 as represented in Table D10 (Appendix D) and saved huge amount of money and time. Graphical presentation of workload contribution of ELISA and LC-MS/MS from 2010 to 2012 for each matrix type is presented in Fig. F1 to F7 (Appendix F) and Fig.18.

4.3 Summary

The approximate cost of analysing microcystins per samples by LC-MS/MS is 2.7 times higher than ELISA. The cost per year for 657 (average number of samples per year at MOE) samples will be $89,260 if water samples are tested by only ELISA and $240,000 if they are tested by only LC-MS/MS. This is approximately a saving of $150,740 per year. By this rationale, ELISA would have saved $452,220 over the period of three years (2010 to 2012).

Further reduction in the LC-MS/MS work load could be achieved in the future by substituting the testing of both type of water samples (WD & WS) by ELISA and also educating the clients regarding the fitness of ELISA for the client's purpose**.**

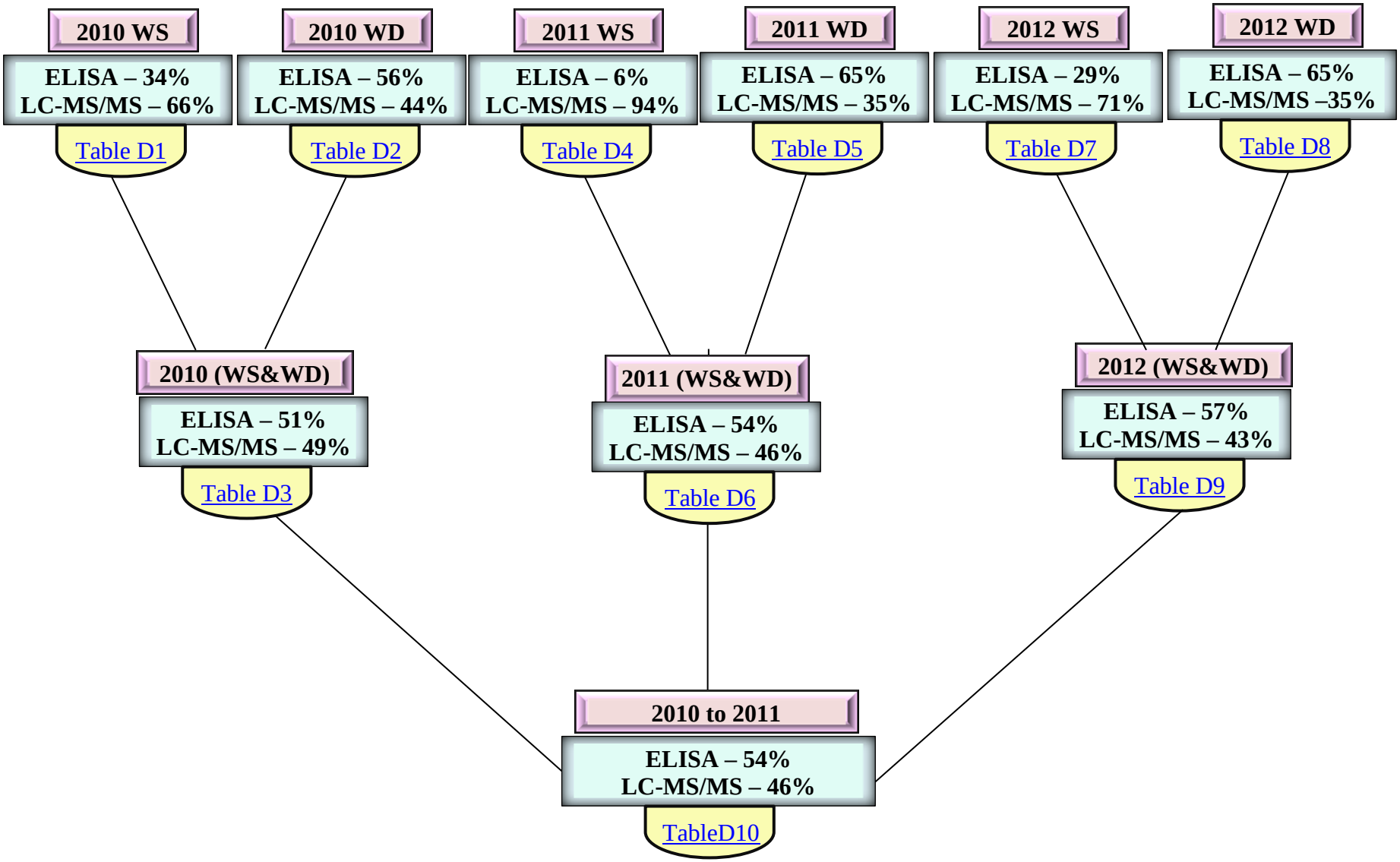

FIGURE 18. SUMMARY OF WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS FROM 2010 TO 2012

5 Experimentation

This research is comprised of two types of experiments – ELISA and PP2A. First, ELISA was performed on the water samples to determine the concentration of total microcystins. Later on, PP2A will be performed on selected samples for toxicity analysis. The results obtained from PP2A and ELISA will be sorted on the matrix (WS, WD) type. A correlation between ELISA and PP2A results will be plotted for each matrix (WS & WD). The sample preparation and the details of these both methods are as follow:

5.1 Sample Source and Preparation

The surface and drinking water samples from 2010 to 2012 were collected through the LaSB of MOE under the "Drinking water surveillance program" (DWSP) and MOE "Field Operation Division" for the routine analysis of total microcystins. The samples were stored at -20ºC at Laboratory Services Branch, Etobicoke.

The samples selected for study were positive either by LC-MS/MS method or ELISA, or they were positive by both. These samples were frozen and thawed twice to perform the ELISA and PP2A. In total, 31 surface water samples (Table 44) and 44 drinking water samples were selected (Table 46).

To analyse exotoxin and endotoxin components of heptapeptide microcystin, some kind of cell disruption method is required. Hence, samples were freeze thaw twice (Rapala *et al.* 2002) to lyse the cell and release intracellular microcystins prior to PPIA and ELISA.

5.2 ELISA

"Microcystins-ADDA ELISA (Microtiter Plate)" Product No. 520011 manufactured by Abraxis was employed to detect total microcystins in the selected samples. This ELISA kit is based on the principle of indirect competitive ELISA. Microcystins and nodularins in water samples can be detected by this quantitative, sensitive, and variant-independent assay. The detection limit of this ELISA is 0.10µg/L (ppb).

5.2.1 Material

This Abraxis kit includes: one microtiter plate (12 X 8 strips) coated with an analog of microcystins conjugated to a protein, one control (0.75 ± 0.185 ppb), eight standards of microcystin-LR at various concentrations (0, 0.05, 0.10, 0.20, 0.40, 1.0, 2.0, 5.0 µg/L), one sample diluent, one antibody solution, one anti-sheep-HRP (horse radish peroxidase) conjugate solution, one wash Solution (5X), one substrate (color) solution (TMB) and one stop solution. Additional materials not supplied by manufacturer, includes one micro-pipettes with disposable plastic tips (20-200µL), one multi-channel pipette (50-300µL), deionized or distilled water, paper towel, timer, parafilm and microtiter plate reader (wavelength 450 nm).

5.2.2 Principle of ELISA

The test is based on indirect competitive ELISA (Fig.19).. Microcystins/nodularins, if present in the sample, are allowed to bind to anti-microcystins/nodularins antibodies so that the binding sites on the antibodies become preoccupied. After that, the mixture is added to a microtiter plate, which has been coated with a microcystins-protein analogue. This protein analogue is presumed to be biotinylated microcystin-LR.

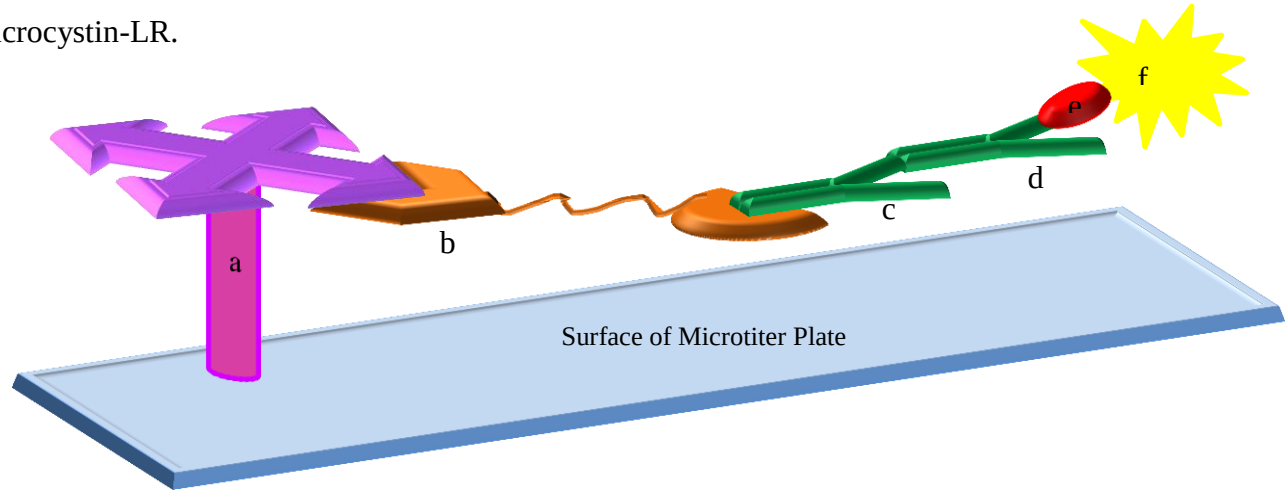

FIGURE 19. PRINCIPLE OF INDIRECT COMPETITIVE ELISA FOR THE DETECTION OF MICROCYSTINS

a-avidin/streptavidin; b- biotinylated microcystin-LR; c- Anti-microcystins antibodies; d-secondary antibody; e-HRP; f-substrate

This assumption is based on the popular protein immobilization technology of using biotin to derivatize the target protein (microcystins) followed by capturing the derivatized proteins by

avidin/streptavidin. Avidin/streptavidin is previously immobilized on the plate. Avidin/streptavidin binds strongly to polystyrene microtiter plate. Biotin and avidin/streptavidin constitute a strong, covalent binding pair thereby immobilizing the biotinylated protein. Furthermore the streptavidin arm ensures the derivatized proteins are displayed and accessible to the antibodies. Anti-microcystins antibodies with binding sites not yet occupied can now bind to the protein analogue immobilized on the coated plate. A secondary antibody conjugate (Anti-sheep IgG-HRP) is added. Finally, addition of a chromogenic substrate solution produces a color reaction. The intensity of the color is inversely proportional to the concentration of microcystins present in the sample i.e. darker color indicates lower microcystins concentration; lighter color indicates higher.

5.2.3 Method

Abraxis ELISA ADDA kit is routinely used at MOE for the detection of total microcystins. The MOE method code is MCYST-3469. The same product (kit) from the manufacturer was used from 2010 to the present time, and is also used in this research.

Detailed description of the method can be found in the Appendix G and is also available online at http://www.abraxiskits.com/uploads/products/docfiles/279_Microcystins%20ADDA%20ES.pdf
The indirect competitive ELISA was performed as per manufacturer instruction (Fig.20). Briefly, 125µl of the standard solutions, control or samples were dispensed into designated wells of a blank plate in duplicates or triplicates. Prior to the experiment, the well positions of each sample or standard were assigned in a plate map by designed. Equal volume of 125µL of antibody solution was added to the wells and the plate was incubated for 30 minutes. Note: all incubations of the plates were at room temperature on a shaker with a lid covering the plate. After incubation, 100µl of solution from each well of the blank plates was transferred to the microcystins analogue-coated plate and incubated for 90 minutes. The plate was washed three times with 1X washing buffer. After washing, 100µL of enzyme conjugate solution was added to the wells and the plate was incubated for 30 minutes. The plate was washed again three times with 1X washing buffer. Substrate solution (100µL) was added and the plate was incubated for 30 minutes. Finally, 50µL of stop solution was added and the absorbance was measured in a plate reader (SPECTRAmaxPlus384) at 450nm.

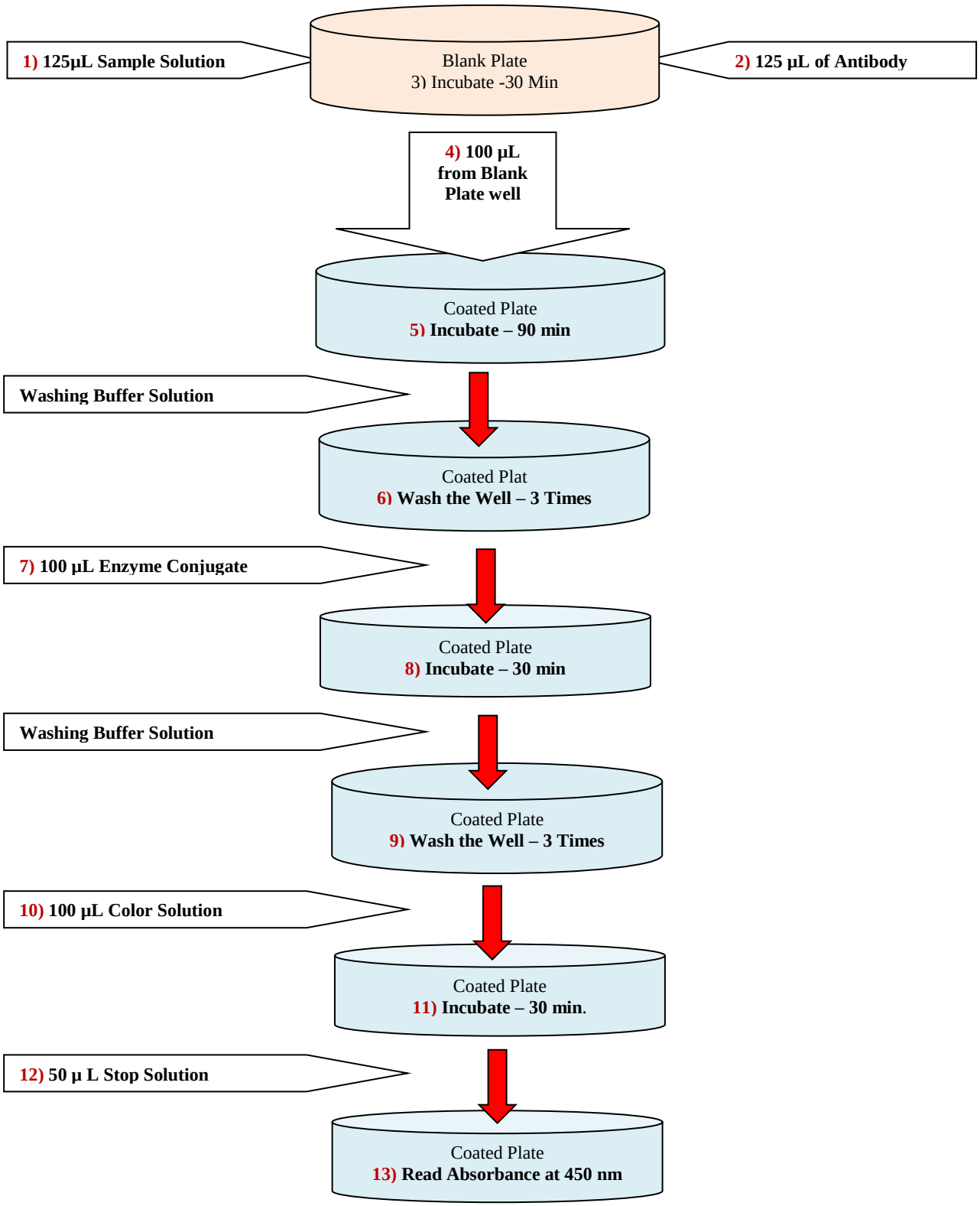

FIGURE 20. FLOW CHART OF MICROCYSTINS ELISA PROCEDURE

A calibration curve is constructed after capturing the absorbance by using commercial software (SoftMaxPro). The program allows plate map design. The operator exports the absorbance values in text format, which is imported to excel work sheet. After recording the measured absorbance, or optical density (OD), of each well, the average OD of each set of calibrators or samples is calculated. Then using the average OD, percentage of Baseline zero ($\%B_0$) is calculated. $\%B_0$ is the parameter which indicates the amount of microcystins in each well, relative to the negative control (NC) wells. The following formula is used to calculate the $\%B_0$

$$\%B_0 = \frac{(average\,OD\,of\ calibrator\ or\ sample) * (100)}{average\,OD\,of\ Negative\,Control}$$

The Baseline zero ($\%B_0$) (y-axis) is graphed versus microcystin concentration (x-axis) using a semi-log plot. The "solver" function in MS excel is used to calculate the squares of differences between actual and calculated curves used by Solver function. Solver function also minimize the "sum of squares of the differences" between Ypred (normalized ELISA response= OD as %of negative control OD) and Y. If the difference is more than 31 the experiment run is rejected (Figure 21).

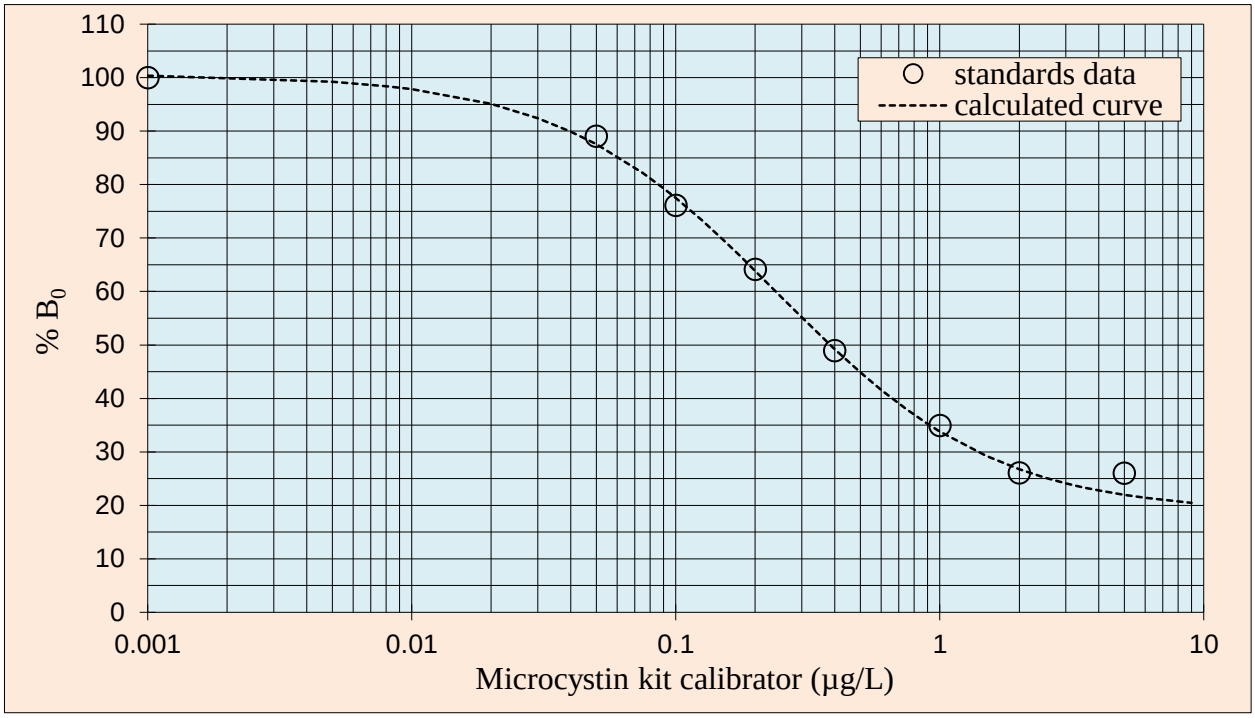

FIGURE 21. ELISA CALIBRATION CURVE BY FOUR PARAMETER FITTING

5.3 PP2A

Protein phosphatase inhibition assay (PPIA) is based on the measurement of protein phosphates enzyme inhibition activity by microcystins. There are many types of protein phosphates such as Type PP1, PP2, PP4, PP5, PP6 and PP7. PP2 have two subtypes called PP2A and PP2B. Two of these enzymes commonly used in PPIA are PP1 and PP2A. The enzyme used in this kit {Microcystins/Nodularins PP2A-Product No. 520032 by Abraxis (manufacturer)} is protein phosphates subtype 2A. Hence, the assay was named by the manufacturer as PP2A (protein phosphatase 2A). The term "PP2A" will be used instead of PPIA henceforth. The MDL of PP2A (Abraxis) is 1.0µg/L.

5.3.1 PP2A Principle

Inhibition activity of phosphatase enzyme 2A (PP2A) by microcystins/nodularins is measured (Fig.22). Sample is incubated with protein phosphatase enzymes (PP2) at 37ºC.

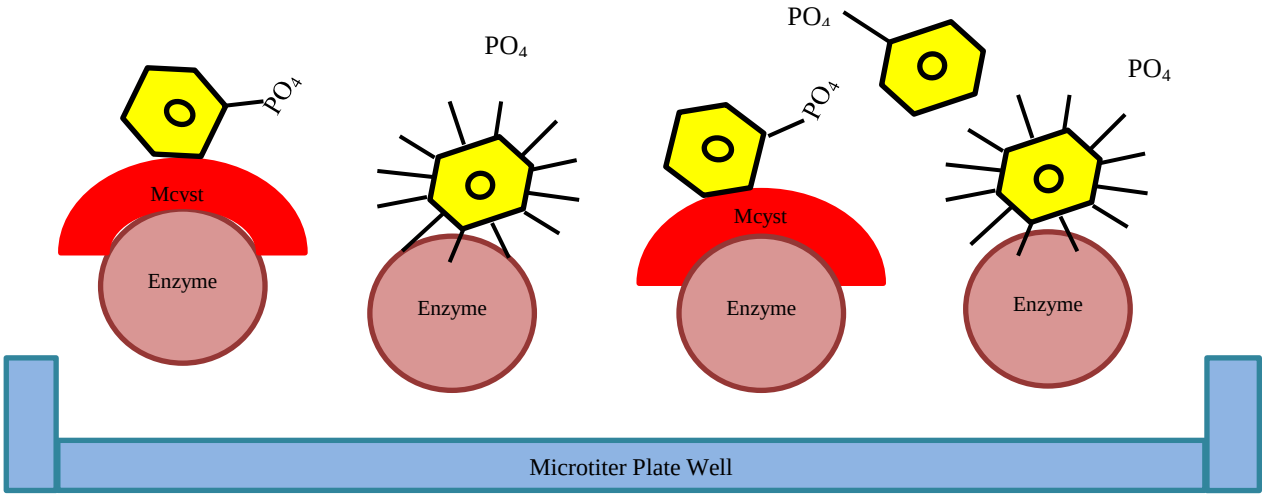

FIGURE 22. PRINCIPLE OF PROTEIN PHOSPHATASE INHIBITION ASSAY

Microcystin, if present in the sample, binds to protein phosphatase enzyme. A substrate is added which is hydrolysed by PP2 enzyme and produces a color. The absorbance of the color is measured at 405nm with a plate reader. Inhibition activity of the PP2A enzyme is proportional to the amount of microcystins present in the sample. The concentration of the toxin in the sample can be calculated using a standard curve.

5.3.2 Material

Materials provided in the kit includes: one 96-well microtiter plate (Fig.23), four vials of phosphatase, four standards of microcystin-LR at various concentrations 0.25, 0.50, 1.00, 2.50 µg/L, one vial of chromogenic Substrate, one vial of Phosphatase Dilution Buffer and one vial of Stop Solution. Additional materials required for the experiment but not included in the kit were Micro-pipettes with disposable plastic tips (10-200 and 200-1000µL), Multi-channel pipette (50-250µL), Microtiter Plate Reader (wave length 405 nm), Timer, Parafilm, Distilled or Deionized water, Vortex Mixer and incubator at 37 +/- 2 °C.

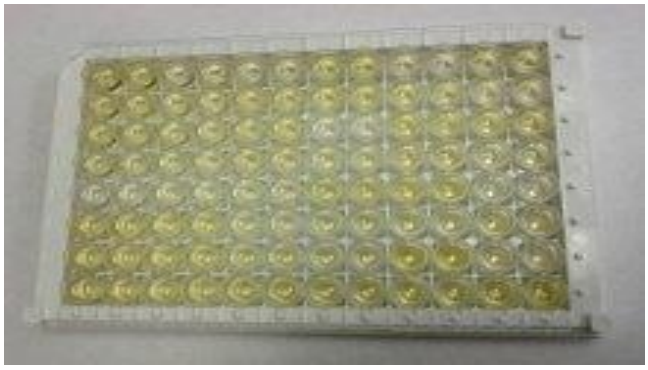

FIGURE 23. MICROTITER PLATE WITH 96-WELLS FOR ELISA

5.3.3 Method

Detailed description of protein phosphatase inhibition assays available online at http://www.abraxiskits.com/uploads/products/docfiles/371_APP2A%20PL%20Users%20Guide.pdf and also can be found in the Appendix H. Briefly, 50µL of each microcystins standard or sample were dispensed into designated wells of a microtiter plate in duplicates. Phosphatase solution (70µL) was added to the each well followed by addition of 90µL of chromogenic substrate. The solution was gently mixed on a shaker for 30 seconds. Adhesive film was applied and plate was incubated for 50 min at 36°C. Finally, after adding a stop solution absorbance of samples and standards were measured at 405nm in a plate reader (SPECTRAmaxPlus384). Flow diagram of PP2A procedure is summarized in Fig.24.

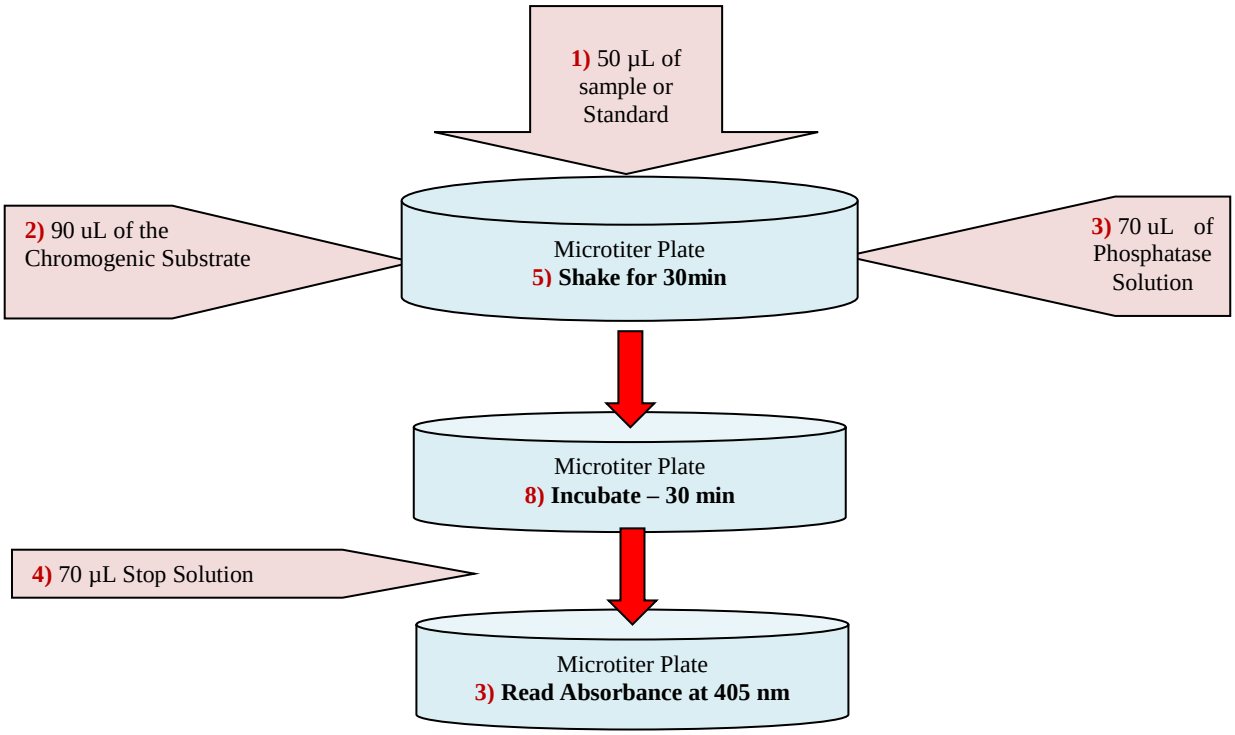

FIGURE 24. FLOW CHART OF MICROCYSTINS PP2A PROCEDURE

Concentrations of the microcystins in the sample were calculated using the same four parameter fitting parameter standard curve (Fig 25) previously used for the calculations of microcystins in the ELISA (Fig.25). The only difference was ELISA kit provides seven standards while PP2A provides four standards and no zero standards. Deionized distilled water was used as a zero standard in this experiment.

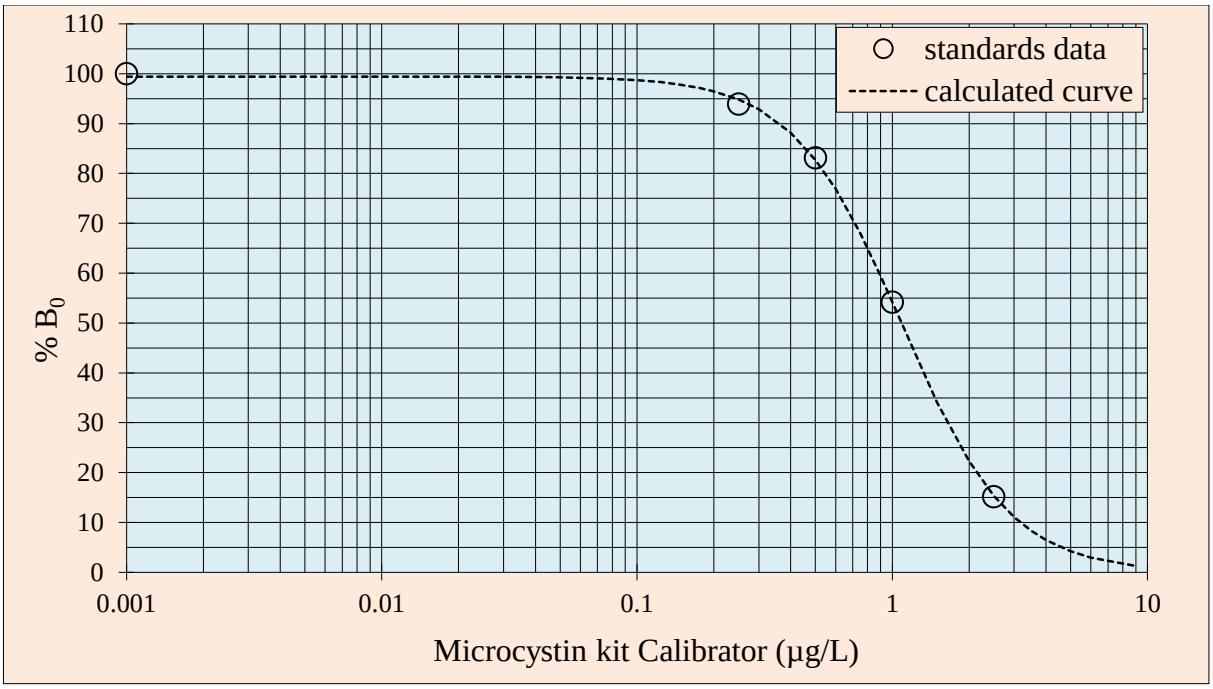

FIGURE 25. PP2A CALIBRATION CURVE BY FOUR PARAMETER FITTING

5.4 Results of ELISA and PP2A

The ELISA procedure is already established and used in routine at MOE. The newly available PP2A was performed first time. Initial results indicated there is room for improvement. Therefore, assay optimization experiments were performed as follow.

5.4.1 Protein Phosphatase Inhibition Assay Optimization

PP2A was optimized by investigating various incubation times at 36^{o}C. The results are shown in the Fig.26, which is a plot of absorbance (of para-nitro-phenol phosphate) versus concentration (of microcystin-LR). The manufacturer's recommended 30min incubation produced a shallow curve which makes the assay not very sensitive (red line). Optimal incubation period of 55 minutes was chosen because the slope (green line) is most distinct. Furthermore, absorbance higher than 2.5 OD units is generally less accurate due to the limitation of the spectrophotometer optical systems. Our optimized method dramatically improved the signal-to-noise differential resulting in more accurate quantitation. The differential at 55min is 2.29 OD units while the differential at 30min is only 1.38 OD units.

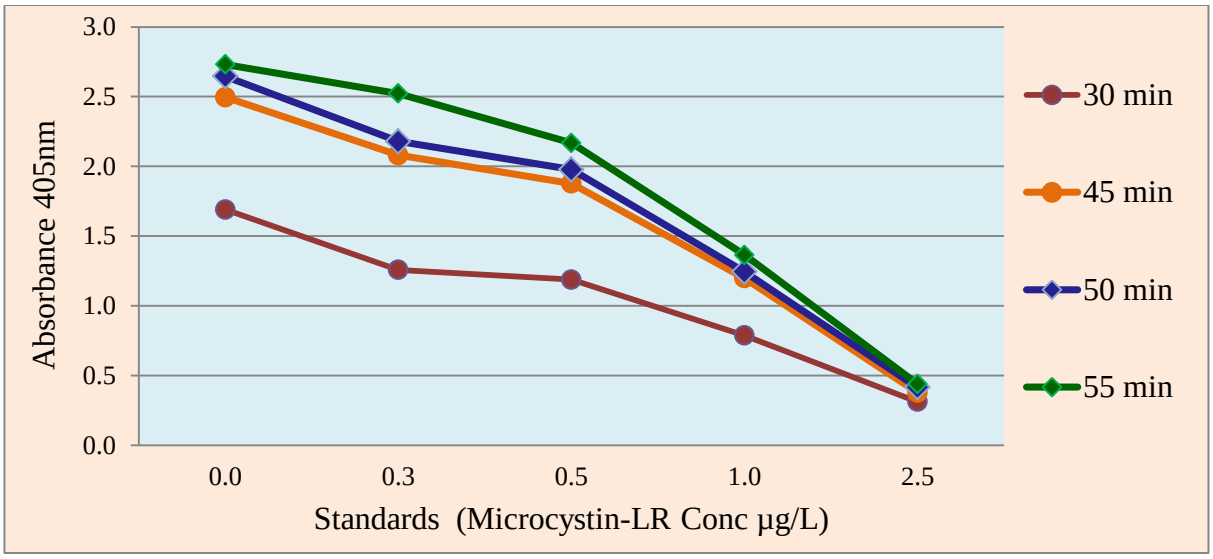

FIGURE 26. OPTIMIZATION OF PP2A BY EXTENDED INCUBATION TIME AT 36ºC

The effect of shaking versus standing during incubation was also investigated. The results (Fig.27) show that shaking did not make any difference on PP2A results but prolonged incubation time did. Based on these observations shaking at 36ºC was not investigated.

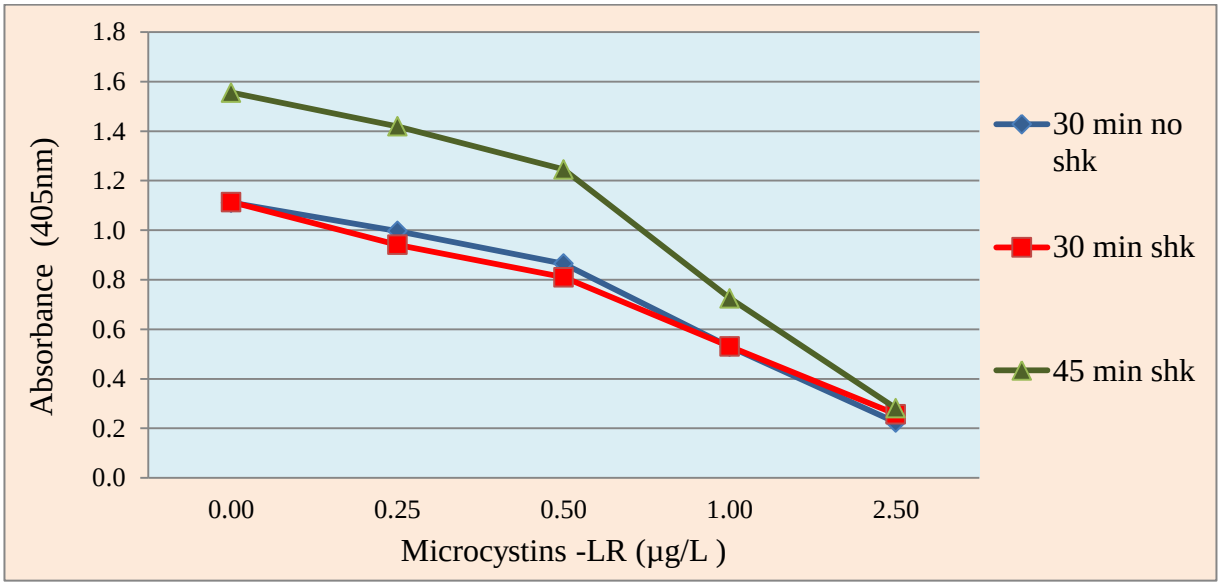

FIGURE 27. PP2A SHAKING VERSUS NO SHAKING AT ROOM TEMPERATURE

5.4.2 Assays Performed on Water Samples

ELISA and PP2A were performed in parallel on selected samples from 2010 to 2012 (Table 44, 46). Samples were freeze thaw twice before testing.

Reproducibility of microcystins ELISA results was examined by comparing results from these runs to the historic results recorded in MOE data base {laboratory information management system (LIMS)}. After 1-2 years of storage at -16ºC, reproducibility is such that surface water samples exhibited an average of 57% (Table 44) of its historic value while drinking water was 54% (Table 46).

In these parallel runs, the average ELISA results were 16 times higher than PP2A for the surface water samples (Fig.28).

5.4.2.1 Surface Water Results

Microcystins were analysed by PP2A and ELISA in 31 selected surface water samples. Samples in counter #1 to #8 were ideal to test the hypothesis of this research. These samples were negative by LC-MS/MS and positive by ELISA in LIMS. PP2A was performed to adjudicate the discrepancy between LC-MS/MS and ELISA results. Samples were frozen from a long period of time; hence ELISA was performed on the same samples once again to find the present (2013) concentration of microcystins in case samples integrity after storage is in question. Comparing the results between ELISA-LIMS and ELISA-2013 there was a 50 to 60% drop in microcystins after 1-2 years of storage (Table 44).

PP2A results were compared with ELISA-2013 and LC-MS/MS-LIMS. PP2A results support ELISA-2013 even after lower recovery of microcystins due to storage. All these eight samples negative by LC-MS/MS and positive by ELISA were also positive by PP2A. Therefore, the PP2A results validated the hypothesis of this research that ELISA detects numerous variants of microcystins, hence produces more true positive results than LC-MS/MS.

There were not enough ideal (LC-MS/MS negative, ELISA positive) samples to test the hypothesis, hence, few more samples were included in the research (counter #9 to #31). Twenty six samples (counter #1 to #26) out of these 31 were previously analysed by both ELISA and LC-MS/MS. Hence, results were available in the LIMS. However, 5 samples in counter #27 to #31 were only analysed by ELISA, therefore no results by LC-MS/MS in LIMS. The percentage calculation in the following paragraphs will exclude the last 5 samples (counter #27 to #31). However, scattered plot performed between ELISA and PP2A includes all samples from Table 44 (n=31).

Results from Table 43 can be divided into four categories. The first category (Counter #1 to #16) describes samples where LC-MS/MS results were either negative or less than the ELISA values in LIMS. Results from ELISA-2013 analysis for the same samples exhibited a lower recovery of microcystins as compare to ELISA-LIMS. Nevertheless, PP2A results are close to ELISA-2013 results. Hence, PP2A results in this category support the ELISA-2013 results.

The second category (Counter #17 to #18) describes a scenario where LC-MS/MS results from LIMS were higher than ELISA. However, ELISA-2013 results are higher than LC-MS/MS. PP2A results support these ELISA-2013 values. Therefore, there is no difference between first and second category.

The third category includes the samples (counter #19 to #21) with LC-MS/MS results lower than ELISA in LIMS. Results by PP2A are significantly higher than ELISA-2013. Perhaps, PP2A inhibitors other than microcystins are present. Especially in the case of counter #21, PP2A results are very much higher than ELISA.

The fourth category comprised of samples where LC-MS/MS results are lower than ELISA in LIMS. PP2A results are very much lower than ELIS-2013. Probably, antibodies in ELISA are identifying the free non-toxic ADDA moiety of microcystins in the samples, hence producing significantly higher results than PP2A.

A linear regression plot was performed between PP2A and ELISA-2013 results to further analyse the correlation between them (Figure. 28). PP2A results were plotted in x-axis and ELISA in y-axis. Four samples (counter #11, #21 #23 #30) in Table 44 had exceptionally high values by both methods.

TABLE 44. SURFACE WATER RESULTS OF MICROCYSTINS FROM LIMS AND PRESENT (2013) EXPERIMENTAION

Counter	Sample #	Matrix	Microcystin Variants (µg/L)				Sum of variants by LC-MS/MS (µg/L)	ELISA LIMS (µg/L)	ELISA 2013 (µg/L)	PP2A (µg/L)
			-LR	-YR	-RR	-LA				
1	WS	C197772-0012	0.05	0.05	0.05	0.05	<MDL	0.85	0.16	0.12
2	WS	C188937-0001	0.50	0.50	0.50	0.50	<MDL	0.55	0.21	0.29
3	WS	C197229-0002	0.05	0.05	0.05	0.05	<MDL	0.80	0.21	0.18
4	WS	C197229-0001	0.05	0.05	0.05	0.05	<MDL	0.85	0.22	0.15
5	WS	C197578-0002	0.05	0.05	0.05	0.05	<MDL	0.55	0.22	0.13
6	WS	C197578-0001	0.05	0.05	0.05	0.05	<MDL	0.50	0.24	0.11
7	WS	C187565-0001	2.50	2.50	2.50	2.50	<MDL	32.70	0.38	0.58
8	WS	C178947-0001	1.00	2.00	2.00	2.00	<MDL	2.80	3.09	1.03
9	WS	C196027-0001	0.52	0.12	0.44	0.52	1.48	2.95	3.11	2.93
10	WS	C197376-0001	0.05	2.70	0.05	0.05	2.70	8.00	4.03	3.02
11	WS	C187436-0001	6.60	3.40	1.00	1.00	12.00	95.60	110	89.65
12	WS	C188064-0001	1.10	0.50	0.50	0.96	2.06	4.50	3.03	2.89
13	WS	C197394-0002	0.24	0.05	0.05	0.18	0.42	0.65	0.15	0.19
14	WS	C197374-0001	0.37	0.05	0.14	0.44	0.95	1.65	0.87	0.92
15	WS	C186621-0001	12	0.12	0.12	12	24.12	63.90	0.94	0.52
16	WS	C197218-0002	0.42	0.05	0.05	0.19	0.61	1.35	1.35	0.80
17	WS	C196029-0001	0.67	0.20	2.00	0.25	3.12	2.30	4.84	4.92
18	WS	C197220-0001	1.40	0.50	0.50	0.34	1.74	1.55	2.11	1.84
19	WS	C197394-0002	0.24	0.05	0.05	0.18	0.42	0.65	0.01	0.26
20	WS	C189075-0001	0.27	0.05	0.05	0.07	0.34	2.05	0.08	0.65
21	WS	C203256-0001	12	0.52	4.40	51	67.92	64.55	62.56	104.91
22	WS	C186812-0001	39	16	4.30	14	73.30	81.60	2.01	0.79
23	WS	C197271-0001	4.20	1.80	0.30	0.11	6.30	10.65	9.03	6.59
24	WS	C197218-0001	0.37	0.05	0.05	0.19	0.56	0.90	0.99	0.61
25	WS	C198208-0001	0.05	0.05	0.05	0.05	<MDL	0.35	0.17	0.00
26	WS	C179269-0001	0.49	0.83	0.83	0.83	0.49	1.20	1.02	0.33
27	WS	C203762-0001	-	-	-	-	-	-	0.20	0.46
28	WS	C203763-0001	-	-	-	-	-	-	0.20	0.97
29	WS	C204635-0001	-	-	-	-	-	-	0.00	0.00
30	WS	C204308-0001	-	-	-	-	-	-	13.63	15.31
31	WS	C204477-0001	-	-	-	-	-	-	0.21	1.16

Although the values between ELISA-2013 and PP2A were in reasonable agreement, these four high values were increasing the weight of the correlation in the scatter plot up to R^2=0.8735 and slope=0.8577(n=31). These four values were outliers and removed from the scatter plot, a strong

correlation is still observed (R^2=0.8155). The slope of 1.168 (n=27) (Fig.28) shows that ELISA results are approximately 17% higher than that of PP2A.

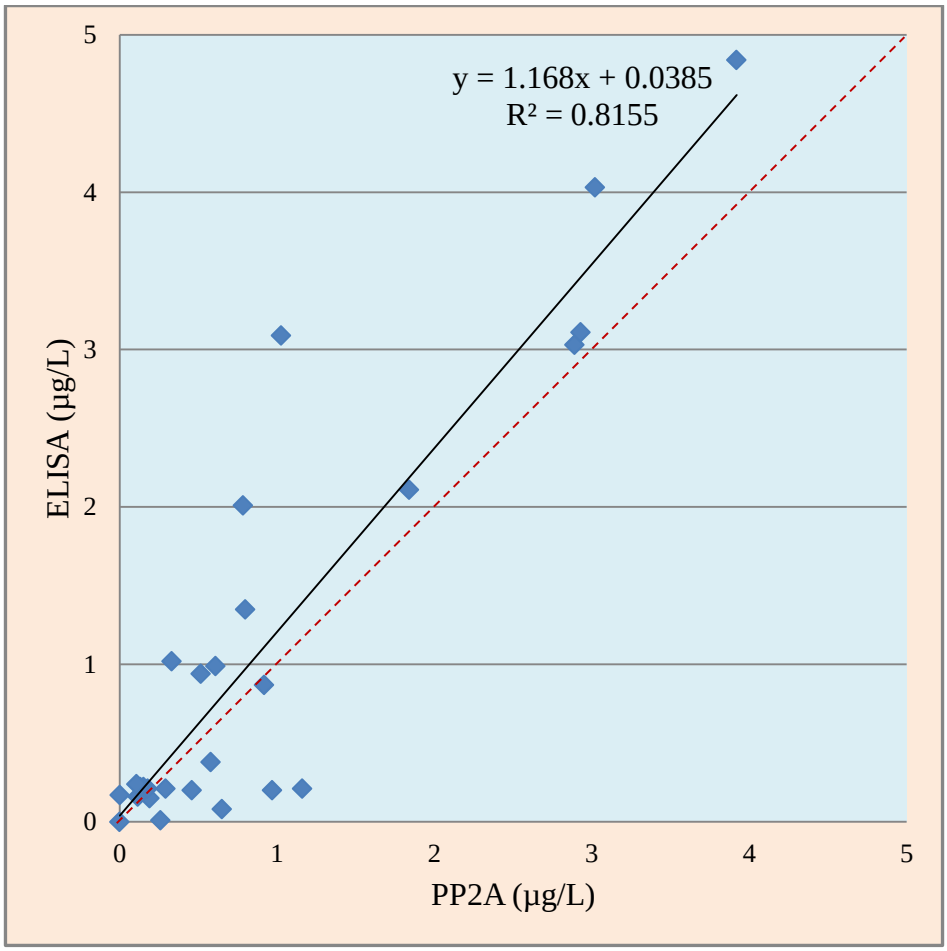

FIGURE 28. WS 2010 TO 2012 - CORRELATION BETWEEN ELISA AND PP2A RESULTS

Fig.28 plots the data in a perfect square with line of idealism at 45º angle. The scatter data points falls more to the left side of the line of idealism, indicating the ELISA values are higher than the corresponding PP2A values. This means ELISA is more sensitive than PP2A.

The surface water results of PP2A presented above were very encouraging for the purpose of measuring the toxicity of microcystins. However, PP2A was found to be unsuitable for colored surface water samples e.g. surface sample # C203886-0001 (Table 46) has very high value of PP2A than ELISA-2013. The assay measures the absorbance of the color. Therefore, the color of a sample

will give false negative results. The picture and the results of one of the brown color sample found during investigations are presented in Fig.29 and Table 46 respectively.

TABLE 45. MATRIX EFFECT ON PP2A FOR THE DETECTION OF MICROCYSTINS IN COLORED SURFACE WATER SAMPLE

Counter	Matrix	Sample #	Microcystin Variants (µg/L) -LIMS				Anatoxin-a (µg/L)	LIMS ELISA (µg/L)	2013 ELISA (µg/L)	2013 PP2A (µg/L)
			-LR	-YR	-RR	-LA				
1	WS	C203886-0001	28	5.0	5.0	5.1	2.0	126.5	50.16	1.57

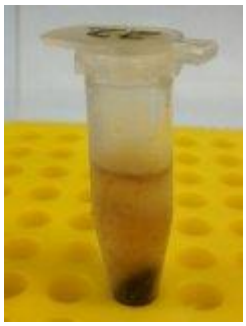

FIGURE 29. SURFACE WATER SAMPLE (C203886-0001) WITH BROWN COLOR

Particularly, water samples from marshes or stagnant are colored. Most of the time, the color intensity can be reduced by centrifugation.

5.4.2.2 Drinking Water Results

PP2A results of drinking water samples are represented in Table 47. PP2A results were 55% higher than ELISA-2013 which is opposite the surface water results (ELISA-2013>17% than PP2A)

TABLE 46. DRINKING WATER RESULTS OF MICROCYSTINS FROM LIMS AND CURRENT EXPERIMENTAION

Counter	Matrix	Sample #	Microcystin Variants (µg/L) –LIMS				Anatoxin-a (µg/L)	LIMS ELISA (µg/L)	2013 ELISA (µg/L)	2013 PP2A (µg/L)
			-LR	-YR	-RR	-LA				
1	WD	C186659-0001	0.08	0.05	0.05	0.05	0.02	0.94	0.001	0.00
2	WD	C197326-0001	0.1	0.05	0.05	0.08	0.02	0.3	0.001	1.18
3	WD	C197443-0001	0.07	0.05	0.05	0.05	0.02	0.2	0.001	0.99
4	WD	C197994-0001	0.05	0.05	0.05	0.05	0.02	2.1	0.001	1.26
5	WD	C197689-0001	0.05	0.05	0.05	0.05	0.02	0.25	0.01	1.22
6	WD	C197331-0001	0.14	0.05	0.05	0.08	0.02	0.3	0.02	0.44
7	WD	C197649-0001	0.07	0.05	0.05	0.06	0.02	0.35	0.02	0.84
8	WD	C187004-0001	0.09	0.09	0.05	0.05	0.02	1.15	0.03	0.00
9	WD	C197864-0001	0.07	0.05	0.05	0.1	0.02	0.25	0.04	1.00
10	WD	C197480-0001	0.05	0.15	0.05	0.05	0.06	0.35	0.06	1.19
11	WD	C198076-0001	0.05	0.05	0.05	0.05	0.02	0.25	0.07	1.21
12	WD	C198333-0001	0.06	0.05	0.05	0.05	0.02	0.55	0.07	1.23
13	WD	C197822-0001	0.05	0.05	0.05	0.05	0.02	0.3	0.12	1.10
14	WD	C197369-0001	0.05	0.09	0.05	0.05	0.02	1.5	0.15	0.38
15	WD	C198330-0001	0.07	0.05	0.05	0.05	0.02	0.45	0.16	1.20
16	WD	C197656-0001	0.05	0.05	0.05	0.05	0.02	0.9	0.19	0.97
17	WD	C186479-0001	0.05	0.05	0.05	0.05	0.02	0.35	0.25	0.19
18	WD	C197741-0001	0.06	0.05	0.08	0.05	0.02	0.45	0.26	0.25
19	WD	C197334-0001	0.11	0.05	0.22	0.05	0.02	0.6	0.28	0.58
20	WD	C197328-0002	0.08	0.05	0.1	0.05	0.02	0.2	0.3	0.56
21	WD	C197924-0001	0.05	0.09	0.05	0.05	0.02	1.4	0.3	0.44
22	WD	C197332-0001	0.32	0.05	0.37	0.05	0.02	2.1	0.4	1.34
23	WD	C197884-0001	0.11	0.05	0.12	0.05	0.02	1.4	0.41	1.33
24	WD	C197691-0001	0.14	0.05	0.13	0.06	0.02	1.05	0.46	0.89
25	WD	C197690-0001	0.12	0.05	0.14	0.05	0.02	0.65	0.48	0.40
26	WD	C198389-0001	0.05	0.16	0.05	0.05	0.02	3.3	0.48	1.30
27	WD	C198075-0001	0.14	0.05	0.1	0.05	0.02	0.6	0.53	1.35
28	WD	C197821-0001	0.19	0.05	0.21	0.05	0.4	1.3	0.6	1.07
29	WD	C197479-0001	0.15	0.05	0.15	0.06	0.17	0.75	0.69	0.70
30	WD	C198190-0001	0.05	0.12	0.05	0.05	0.02	0.95	0.7	0.51
31	WD	C197481-0001	0.24	0.05	0.16	0.05	0.02	0.8	0.73	0.51
32	WD	C197333-0001	0.57	0.07	0.48	0.05	0.02	0.95	0.86	1.46
33	WD	C197482-0001	0.32	0.06	0.27	0.06	0.02	1.15	1.19	1.38
34	WD	C198546-0001	0.05	0.05	0.05	0.05	0.02	0.2	0.18	1.18
35	WD	C198740-0001	0.05	0.05	0.05	0.05	0.02	0.3	0.28	0.3
36	WD	C198804-0001	0.05	0.29	0.05	0.05	0.02	2.5	2.6	2.42

Counter	Matrix	Sample #	Microcystin Variants (µg/L) –LIMS				Anatoxin-a (µg/L)	LIMS ELISA (µg/L)	2013 ELISA (µg/L)	2013 PP2A (µg/L)
			-LR	-YR	-RR	-LA				
37	WD	C199164-0001	0.05	0.1	0.05	0.05	0.02	0.2	0.19	1.33
38	WD	C203886-0001	0.19	0.016	0.085	0.28	0.02	2.55	2.55	1.99
39	WD	C203977-0001	0.55	0.05	0.033	0.079	0.02	1.8	1.8	0.86
40	WD	C204047-0001	0.033	0.05	0.048	0.027	0.02	0.25	0.25	0.57
41	WD	C203434-0001	-	-	-	-	-	0	0	0
42	WD	C203434-0002	-	-	-	-	-	2.1	2.1	2.58
43	WD	C203434-0003	-	-	-	-	-	7.1	7.1	7.1
44	WD	C203434-0004	-	-	-	-	-	10.25	10.25	8.76

A scattered plot between ELISA-2013 and PP2A (Fig.30) was performed. Six values from ELISA-2013 and four values from PP2A results were found to be outliers. Hence, six samples (36, 38, 39, 42, 43, 44) were excluded from analysis. Scattered plot shows an insignificant correlation between ELISA-2013 and PP2A (n=38, R^2=0.0366, slope=0.1199, p=0.0665).

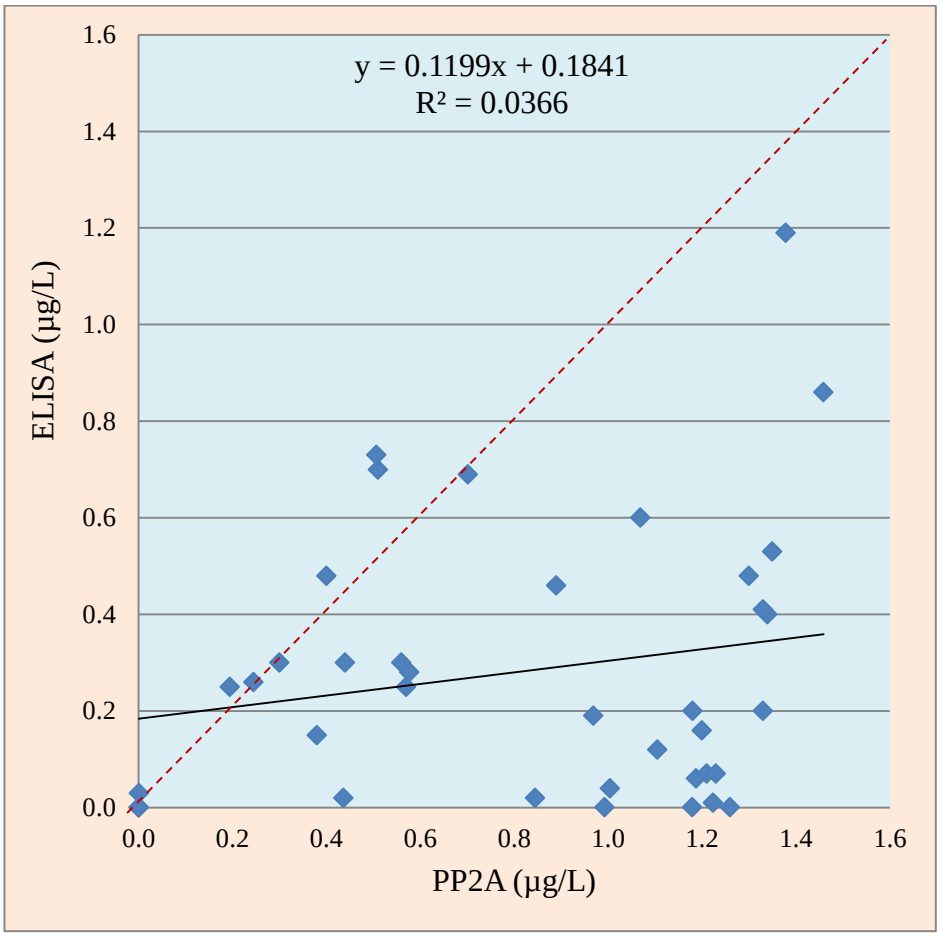

FIGURE 30. WD 2010 TO 2012 – CORRELATION BETWEEN ELISA AND PP2A

Fig.30 plots the data in a perfect square with a line of idealism at a 45^{o} angle. Most of the scatters data points fall to the right side of the line of idealism, indicating that PP2A values are very much higher than the corresponding ELISA values.

Experiments were designed to investigate this lack of correlation and the source of error for the higher PP2A results. PP2A will be positive whenever the PP2 enzyme is inhibited. A significant correlation observed for surface water versus the lack of correlation in drinking water indicates a matrix effect. As per manufacturer, any oxidizing agent or $Na_2S_2O_3$ could be inhibitory. All drinking water sample collected for microcystins analysis at MOE have chlorine at various concentrations. These water samples are also analysed for analytes other than microcystins. To preserve the analytes from oxidation, the chlorine is neutralized by $Na_2S_2O_3$. Approximately 30 drops of $Na_2S_2O_3$ at stock concentration of 25% (W/V) is added to one liter of water sample so that the final concentration is 0.0375% (W/V).

To confirm whether chlorine or $Na_2S_2O_3$ is inhibiting the protein phosphatase enzyme activity, experiment was performed using deionized distilled water (DDW), distilled water (DW), tap water and tap water with 0.001% of $Na_2S_2O_3$ (25%W/V). $Na_2S_2O_3$ was used to neutralize the chlorine. The results clearly indicated that tap water supresses the enzyme activity from 2.5 OD units (DDW and DW) down to approximately 1.6 OD units (Fig.31). This implies chlorinated water inhibited the protein phosphates enzyme to some extent. If this were true, neutralizing the chlorine with sodium thiosulphate ($Na_2S_2O_3$) should return the enzyme activity to normal. This was not the case in Fig.31. The inhibitory effect of $Na_2S_2O_3$ was more prominent than chlorine in DDW.

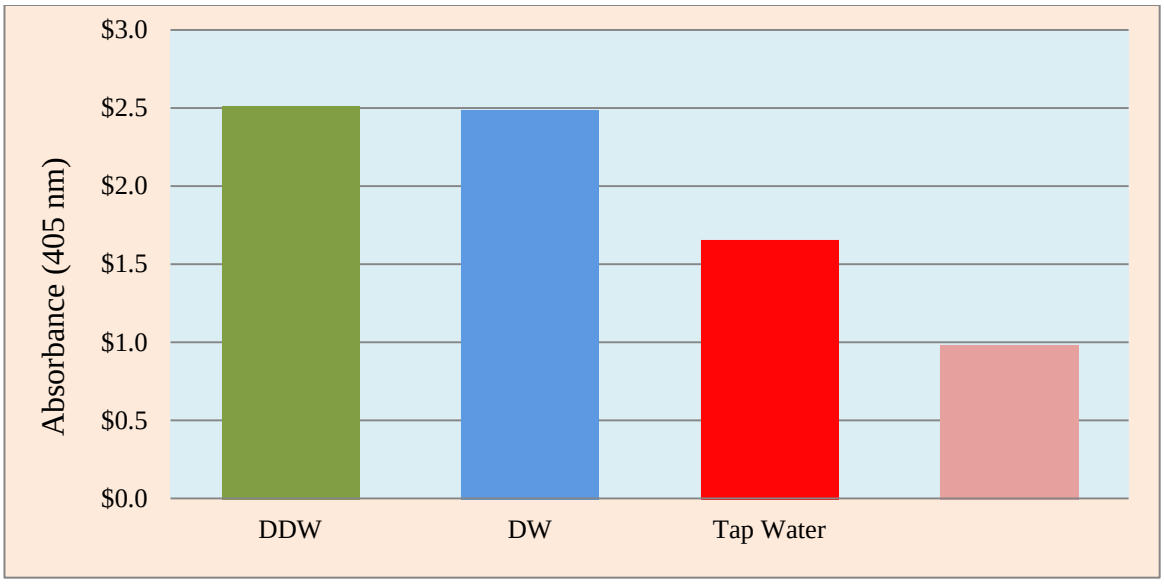

FIGURE 31. COMPARISON OF PROTEIN PHOSPHATES ACTIVITY

$Na_2S_2O_3$ inhibitory effect was further established by a dose-response experiment using five different concentrations (3.75%, 0.375%, 0.0375%, 0.00375%, 0.0003.75%) of $Na_2S_2O_3$ (25%W/V) in DDW. The higher the concentrations of $Na_2S_2O_3$, the higher were the false positive results of microcystins-LR (Fig.32, Fig.33).

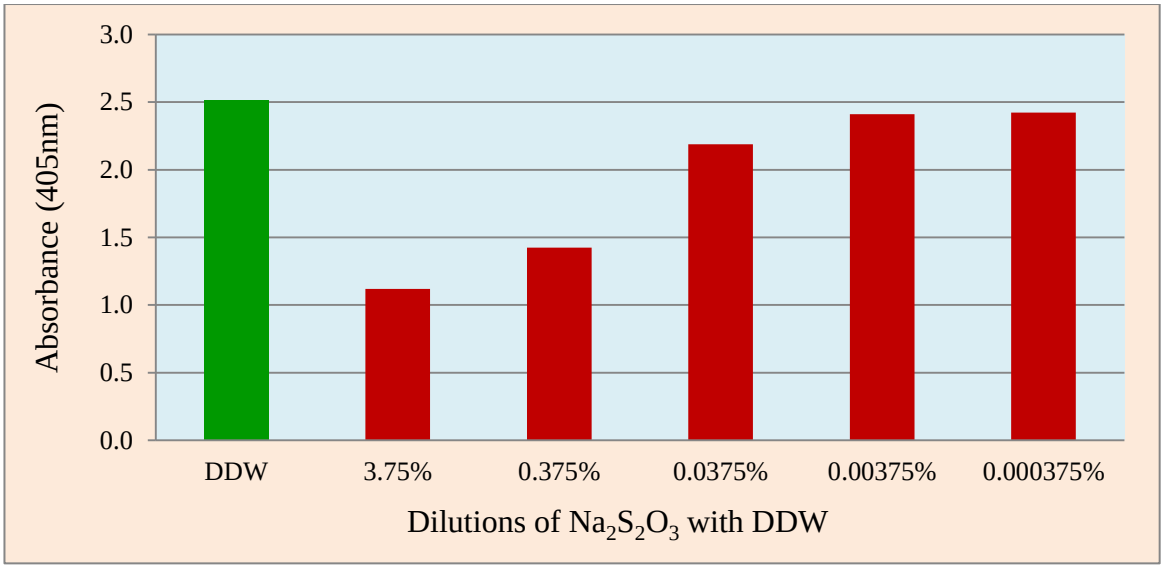

FIGURE 32. MATRIX EFFECT OF $NA_2S_2O_3$ IN DDW ON PROTEIN PHOSPHATASE 2A ENZYME ACTIVITY – ABSORBANCE (nm)

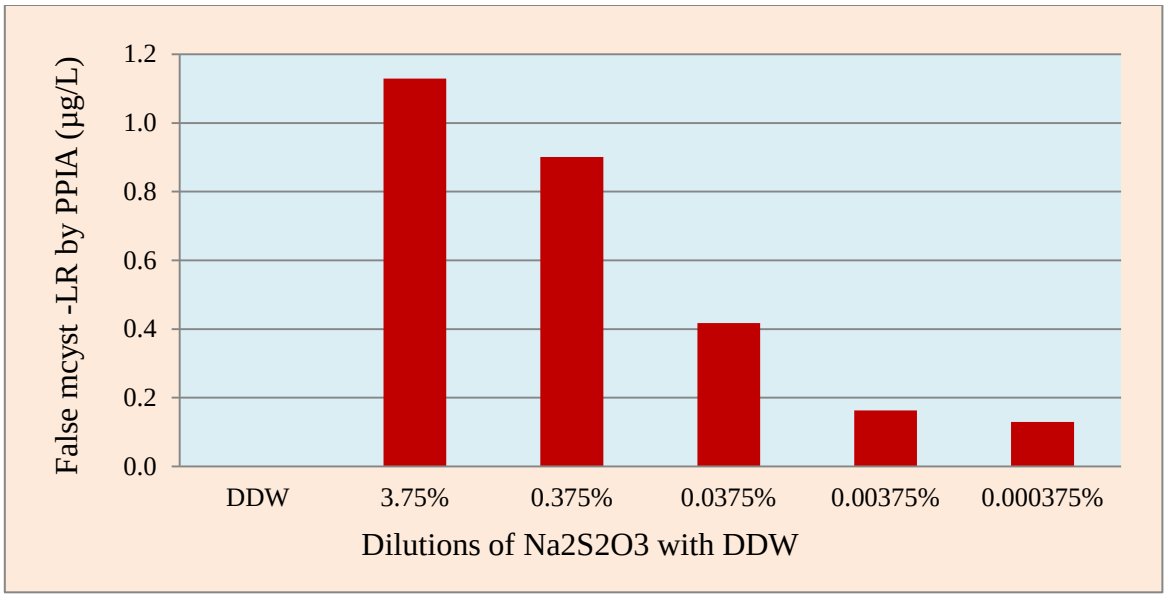

FIGURE 33. MATRIX EFFECT OF $NA_2S_2O_3$ IN DDW ON PROTEIN PHOSPHATASE 2A ENZYME ACTIVITY – CONCENTRATION (µg/L)

Matrix effect on the recovery of microcystin-LR was also investigated. Four different concentration of microcystin-LR (0.125µg/L, 0.25µg/L, 0.5µg/L, 1.25µg/L) and a blank (DDW) were spiked with three concentrations (0.375%, 0.0375%, 0.00375%) of $Na_2S_2O_3$. False results of microcystin-LR recovery were found. The inhibitory effect of $Na_2S_2O_3$ was more prominent in DDW compared to the presence of microcystin-LR in DDW.

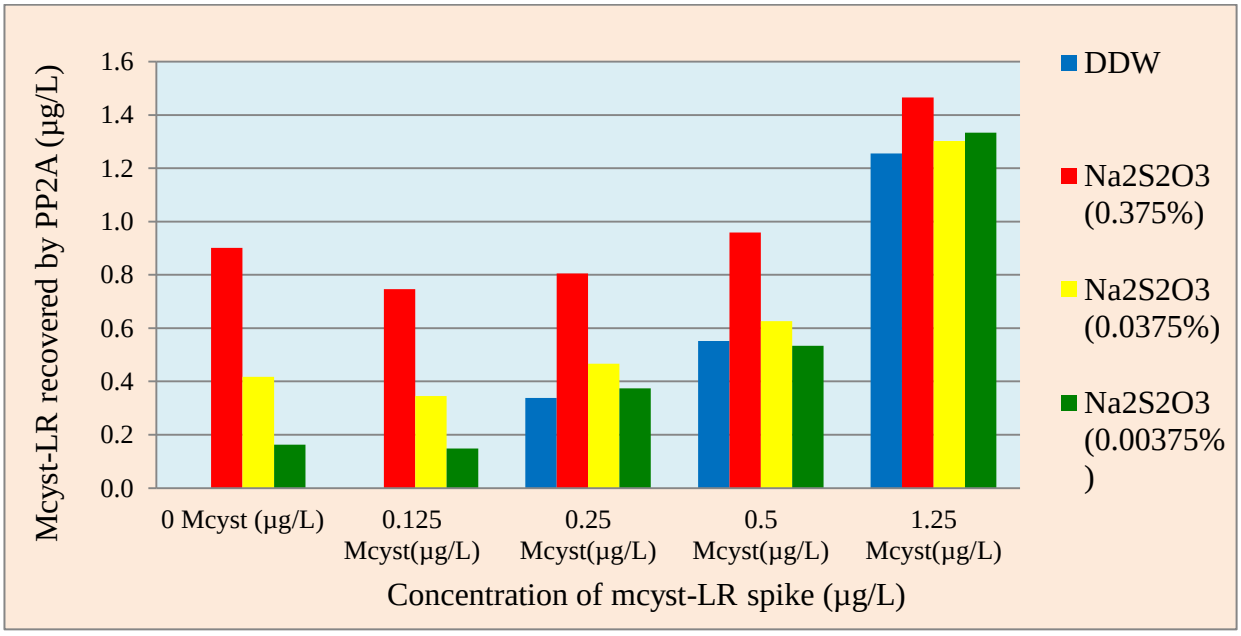

FIGURE 34. MATRIX EFFECT OF $NA_2S_2O_3$ ON THE RECOVERY OF MCYST-LR BY PP2A – ABSORBANCE(nm)

Hence, one clear conclusion can be made that in presence of $Na_2S_2O_3$ in any concentration in water will inhibit the protein phosphatase enzyme (Fig.34). Hence, PP2A results for drinking water results cannot be relied upon.

However, in case of three samples (counter #42 to #44) in Table 47, PP2A was able to detect accurately microcystins. These four samples were quality control samples. Probably samples does not contain any chlorine and sodium thiosulphate, hence PP2A produced results which are comparable with ELISA-2013. .

5.4.3 Summary

The significant findings on studying PP2A and ELISA in parallel are as follows.

PP2A was found to have an MDL range from of 0.25µg/L to 2.5µg/L. Negative samples by LC-MS/MS and positive by ELISA were found to be positive by PP2A too. PP2A results support ELISA results. Hence, the hypothesis that ELISA produces more true positive results than LC-MS/MS because it can detect more variants than LC-MS/MS was found to be true. An acceptable correlation was found between PP2A and ELISA results based on the high R^2 (0.8155) value in surface water samples. PP2A was found to be a reliable method for the detection of microcystins and can be considered as a tier-2 test. However, PP2A is confounded when the surface water samples is colored, especially yellow to brown color. In such cases PP2A results will be unreliable.

The results of PP2A were erroneously higher than ELISA in drinking water samples due to chlorine inhibition of PP2 enzyme. Neutralization of chlorine with sodium thiosulfate did not solve the problem because neutralizer itself is inhibitory to the enzyme.

Hence, it can be concluded that ELISA does not produce false positive results. PP2A is a good probe for none colored surface water but ineffective for the detection of microcystins in treated drinking water samples.

6 Discussion

Increased eutrophication of water bodies has elevated the health risks associated with cyanobacteria worldwide. This situation increases the demand to find faster, reliable and economical methods for monitoring microcystins. From 2010 to 2012, the average number of samples collected per year is 657 at MOE. The total cost per sample by ELISA is $136 and $365 by LC-MS/MS. If all the samples would have been analysed by ELISA, the cost per year would be $89,260 versus $240,000 by LC-MS/MS alone. The kit cost per sample for PPIA is $16 and $10 for ELISA. If ELISA is used as a tier-1 test followed by PPIA as a tier-2 test, PPIA would theoretically cost $16 x 657 = $13,664. In practice, the tier-2 test would need to be run every week, requiring 36 strip wells of a microtiter plate. Over the 26 weeks of warm season, the number of strips required would be 26 x 36 wells = 936 wells = 117 strips (8-wells per strips) = 117/12 kits (12 strips per kit) = 9 kits = $5,400 per year. Hence, the 2-tier test system would cost $5,400+$89,260=$94,660 per year which is still 2.5 times less than LC-MS/MS. More importantly, this 2-tier test system could potentially replace LC-MS/MS if the regulation is changed to monitor total microcystins and disregard microcystin-LR as the sole indicator. This would mean approximately annual savings of $145,340.

Other than economic impact, the major advantage of these bioassays over LC-MS/MS is their turnaround time. To monitor our water resources and to prevent the public from harmful impacts of microcystins in real time, a rapid method is desirable. PP2A can be performed within one and half hour, ELISA in three hours but LC-MS/MS usually needs one week.

In a day, 36 samples can be included in one PP2A run or one ELISA run. On the other hand LC-MS/MS is limited to 20-26 samples per week. The workload capacity, cost and turnaround time of ELISA or PP2A is obviously advantageous. Therefore, ELISA and PP2A are the methods of choice for analysing microcystins in the water.

ELISA is already an established method for the detection of microcystins in drinking water samples (EPA USA, 2010b; Geis-Asteggiante Lucía *et al.*, 2011; Pírez, Macarena *et al.*, 2013). ELISA results as compare to HPLC were (on an average 2.5 times) higher in a study by Tillmans *et al*, (2007) and 7.4 times higher in a study by Conti *et al*, (2006). This study found ELISA was 2.5 times higher than LC-MS/MS from 2010 to 2012 in 30% (215) of samples out of 854 samples. In these three years,

ELISA missed only 4 samples out of 854 samples (0.5%). Over the three years, ELISA consistently showed slightly higher results or more positive results compare to LC-MS/MS. This general trend was observed by numerous workers cited in Table 7, Group 1 and Group 2. The most logical explanation for this trend is that ELISA cross react with multiple variants of microcystins, some of which escaped LC-MS/MS detection. LC-MS/MS is limited to detect only 4 to 5 variants of microcystins, hence the reason for lower results. The one study in Group 3 is out of line with this general trend; this study was performed on food supplement pills and the reason for the lower ELISA results was probably due to poor or inadequate sample extraction from the pills. The present research on 854 samples over three years is the largest and most comprehensive sample size ever reported, based on which a more reliable trend of ELISA versus LC-MS/MS is deemed more credible.

PP2A detects the bioactive toxicity of microcystins (Carmichael & An., 1999). PP2A is sensitive to the toxic microcystins variants. However, the toxic potency differs among variants. Furthermore, the toxicity of the majority of variants is unknown. ELISA detects the total microcystins or structural component of microcystins. Part of this research was designed to evaluate which assay, PP2A or ELISA, will be the more suitable tier-1 assay. Surface water samples testing showed that ELISA results were 17% higher than PP2A. Since PP2A is less sensitive than ELISA, PP2A cannot serve as a tier-1 assay.

Chlorine was found to be inhibitory of protein phosphatase enzyme in treated drinking water samples. Most of the studies found calyculin A and okadaic acid are the inhibitor of protein phosphatase enzyme (Lin *et al.*, 1994; Metcalf *et al.*, 2000). No literature was found on chlorine inhibition of protein phosphatase enzyme. The reason is that these studies were conducted either on spiked samples or laboratory grown cyanobacteria or on surface water samples but not on drinking water samples. Since the aforementioned samples did not contain chlorine, it is not surprising that the inhibitory effect of chlorine on the protein phosphatase enzyme has not yet been reported in the literature.

LC-MS/MS could analyse only four variants (-LR, -RR, -LA, -YR) until 2102. Since 2013, the MOE laboratory has expanded its capability to identify eight more variants (microcystins -LY, -WR, -HtyR, -HilR, -LW, -LF, desmethylmicrocystins-LR, desmethylmicrocystins-RR) due to the recent availability of commercial standards. Agreement (chapter-3) and cost (chapter-4) calculation

performed in this study includes the data from 2010 to 2012. In future studies, the agreement between ELISA and LC-MS/MS could improve with the capability of measuring twelve variants rather than four. However, the advantage of measuring twelve variants for monitoring purposes remains obscure. In contrast, the advantages of ELISA are clear. Furthermore, measuring eight additional variants incurs higher cost for LC-MS/MS. Therefore, the argument that ELISA is more fit-for-purpose than LC-MS/MS remains valid irrespective of how many additional variants LC-MS/MS may able to detect.

Anatoxin-a is rarely found in the water samples examined. Over the period of three years, anatoxin-a was detectable by LC-MS/MS in only 19 samples (19/854 = 2.2%). Among these 19 samples, 9 were excluded due to their unreliable LC-MS/MS results caused by matrix interference. Among the remaining 10 samples (1.2%), only one sample (2.0µg/L) exceeded the proposed guideline of 1.0µg/L (Fawell *et al.,* 1999). Surprisingly, no microcystins was detected by ELISA in this one sample. Unfortunately, this sample from 2010 was not saved for this research. Thus, the disagreement between ELISA and LC-MS/MS results could not be resolved by further testing. Since a receptor binding assay for anatoxin-a (Abraix) is now commercially available, the need for LC-MS/MS for monitoring this toxin is diminished.

For the first time anatoxin-a kit has become commercially available since April 2013. The timing did not allow for the study of this kit within the scope of this research. Nevertheless, investigation of this kit, which is a receptor binding assay, is highly recommended based on the following rationale. First, this kit might provide economical alternative to LC-MS/MS if and when anatoxin-a becomes regulated by *SDWA.* Second, this data base indicated that the rate of occurrence of anatoxin-a is rare (1.2%) over three years. Based on a very small sample size of n=10, no correlation was observed between the concentration of microcystins and anatoxin-a. It would appear that microcystins is not a good predictor of anatoxin-a. Hence, an assay specific to anatoxin-a would be appropriate.

Little is known about the effect of chlorination and boiling on the inactivation of anatoxin-a. Whether anatoxin-a should be regulated, is a complex question beyond the scope of this research.

7 Conclusion

The alarming increase in the frequency and magnitude of cyanobacterial bloom worldwide impose a demand for laboratory analytical methods to monitor the threat to environmental and public health (chapter 2). The traditional LC-MS/MS method is unable to meet this rising demand due to its cost, and cumbersome procedure. The first objective of this thesis is to find solutions to this analytical challenge. To accomplish this objective, a thorough comparison between ELISA and LC-MS/MS were undertaken from the point of view of cost effectiveness as well as reliability. This comparative study leads to the conclusion that ELISA supersedes LC-MS/MS in cost effectiveness, workload throughput, turn-around time, ease of operation, lack of matrix interference and reliability of results.
Based on this conclusion it follows that ELISA, not LC-MS/MS should be the first line of defence against blue green algae bloom events. This hypothesis was proposed by the Ryerson research team to initiate the amendment of O. Reg. 248/03, which came into effect in December 2009. The amendment allowed municipal and private laboratory to contribute to the province wide monitoring of drinking water samples while circumventing the requirement for LC-MS/MS capabilities. This was a milestone in the advancement of environmental science and policy hand in hand. By reviewing and analysing a much a larger sample size from 2010 to 2012, this thesis allows the earlier hypothesis of Ryerson research team to mature into a sound theory that ELISA is the tier-1 method of choice based on the criteria of fit-for-purpose (chapter 3) and cost-efficiency (chapter 4). Since 2013, the MOE has increased the LC-MS/MS capability to measure twelve variants. However, the conclusion of this thesis remains unchanged because ELISA supports the *SDWA* requirement cost-effectively.

The second objective of this thesis is to find a suitable tier-2 test to complement ELISA (chapter 5). The motivation for this exercise was two-fold. Firstly, when ELISA and LC-MS/MS results disagree, a third test is required to adjudicate the discrepancy. Secondly, if this third test is fit-for-purpose, then it could potentially replace LC-MS/MS as a tier-2 test candidate. To achieve the second objective, PP2A was chosen, optimized and the assay attributes were characterized. Despite its limitations and shortcomings, PP2A was found to be a suitable tier-2 candidate over LC-MS/MS for surface water.

In summary, this thesis proposed a 2-tier test system: tier-1 ELISA and tier-2 PP2A for surface water samples only. The surface water samples are not regulated under *SDWA*. Hence, replacing of LC-MS/MS with 2-tier test system would be easier than for drinking water samples. This work flow

would be most cost effective and cost efficient. PP2A is not recommended for the detection of microcystins in drinking water due to matrix interference of chlorine and sodium thiosulphate.
The question of anatoxin-a could be addressed by the newly available receptor binding bioassay as a potential replacement for LC-MS/MS.

The Ryerson team had a successful track record in influencing the amendment of O. Reg. 248/03 in 2009 to accommodate ELISA, to empower private and municipal sectors, and to revive the original spirit of the law. This study provides further momentum of technological advancement conducive to additional amendment of the regulations to better protect public and environment health from the threat of cyanobacteria toxins. To this end, the 2-tier test system proposed in this thesis creates a paradigm towards the amendment of O. Reg. 248/03 to accept PP2A which will propel the amendment of *O. Reg. 169/03*, specifically regarding the reversion of drinking water standards from 1.5µg/L of microcystin-LR back to 1.5µg/L total microcystins.

8 Recommendation

Future PP2A experiments are required with addition of different concentrations of chlorine to deionized distilled water to proof that chlorine is inhibitory to protein phosphatase enzyme. This experiment would determine conclusively whether PP2A has a place for testing drinking water samples.

On May 12, 2003, the Ontario Environmental Registry published a Policy Decision statement as cited below.

Registry Number: PA 03E0001

Ministry Reference Number: 2003011501

Title: Proposal to Establish an Ontario Drinking Water Standard for Cyanobacterial Toxins (Microcystin LR)

Policy Statement: The Ministry has adopted the Canadian Drinking Water Guideline (CDWG) for cyanobacterial toxins of 0.0015 mg microcystin per litre as an Ontario Drinking Water Standard (ODWS), as part of the Ontario Drinking-Water Quality Standards Regulation (*O. Reg. 169/03*) under the *Safe Drinking Water Act*, 2002.

http://www.ebr.gov.on.ca/ERS-WEB-External/displaynoticecontent.do?noticeId=MTk3MTU=&statusId=MTk3MTU=&language=en

In view of the above, it is clear that the spirit of the law did not specify microcystin-LR or any specific variants but rather microcystins in total. For some reason, when translated into *O. Reg. 169/03*, the letter of the law became microcystin-LR. Perhaps the regulation was written based on science and technology available at the time, namely LC-MS/MS, which quantifies individual variants. Today, bioassays such as ELISA and PP2A are shedding new light in toxin detection and toxicity measurement. Bioassays have numerous advantages over LC-MS/MS including qualitative (screening test) and quantitative (concentration) detection, low cost, high throughput, fast turnaround time, and commercially available kits. In 2008, the Ryerson team has transferred the ELISA technology to private and municipal laboratories without LC-MS/MS capability to enable them to monitor microcystins in drinking water and surface water locally. Vision of the Ryerson team came to pass in 2009 when O. Reg. 248/03 was amended to empower ELISA to detect microcystins as first response to algal bloom events. This was the first milestone of revising the letter of the law back to

the spirit of the law. Despite this preliminary success, the final goal is not reached yet due to two questions. First, when ELISA is positive and LC-MS/MS is negative, the possibility of a false positive ELISA result must be ruled out in order to avoid issuing drinking water advisory or beach closure prematurely. Second, surface waters are still analysed by the costly LC-MS/MS because they fall outside the drinking water regulation and because of the potential threat of anatoxin-a.

PP2A provides a pragmatic answer to the first question. When PP2A is positive on a water sample which exhibits ELISA positive and LC-MS/MS negative results, the probability of a false positive ELISA is low because the PP2A results indicate the sample is toxic. One could argue that the toxicity might be due to non-specific inhibitors, in which case one would have to assume the variants detected by the ELISA is non-toxic; a highly unlikely possibility. Therefore, PP2A addresses the first question adequately concerning surface water samples. With respect to drinking water samples, untreated raw water samples can be analysed by PP2A as well. The dilemma that chlorine treated drinking water cannot be analysed by PP2A is not as disconcerting as it seems. No significant level of microcystins was ever detected in treated drinking water ever since the monitoring of microcystins began in 2003. Hence, the failure of PP2A to test chlorinated water may be a non-issue in practice. If and when the issue arise, one can fall back on the LC-MS/MS method for support.

Changing the test from LC-MS/MS to PP2A would not only save tens of thousands of dollars but also empower private and municipal sectors to completely fulfill the spirit of the law without LC-MS/MS capability. The second part of the question is concerned with the potential threat of anatoxin-a. This study showed that the potential threat of anatoxin-a is remote (<2%). Furthermore, the commercial receptor binding assay kit might proof to be a suitable replacement for LC-MS/MS.

In summary, it is recommended that ELISA should be the tier-1 test and PP2A tier-2 test to replace the LC-MS/MS for all surface water samples matrices without exception. These recommendations are summarized in a flow chart (Fig.35).

Drinking water samples are regulated by *SDWA.* Under *O. Reg. 248/03,* ELISA is the tier-1 test and LC-MS/MS is the tier-2 test to confirm ELISA positive samples. PP2A cannot be used in drinking

water samples due to matrix interference. Hence, the current strategy of ELISA as tier-1 and LC-MS/MS as tier-2 is suitable.

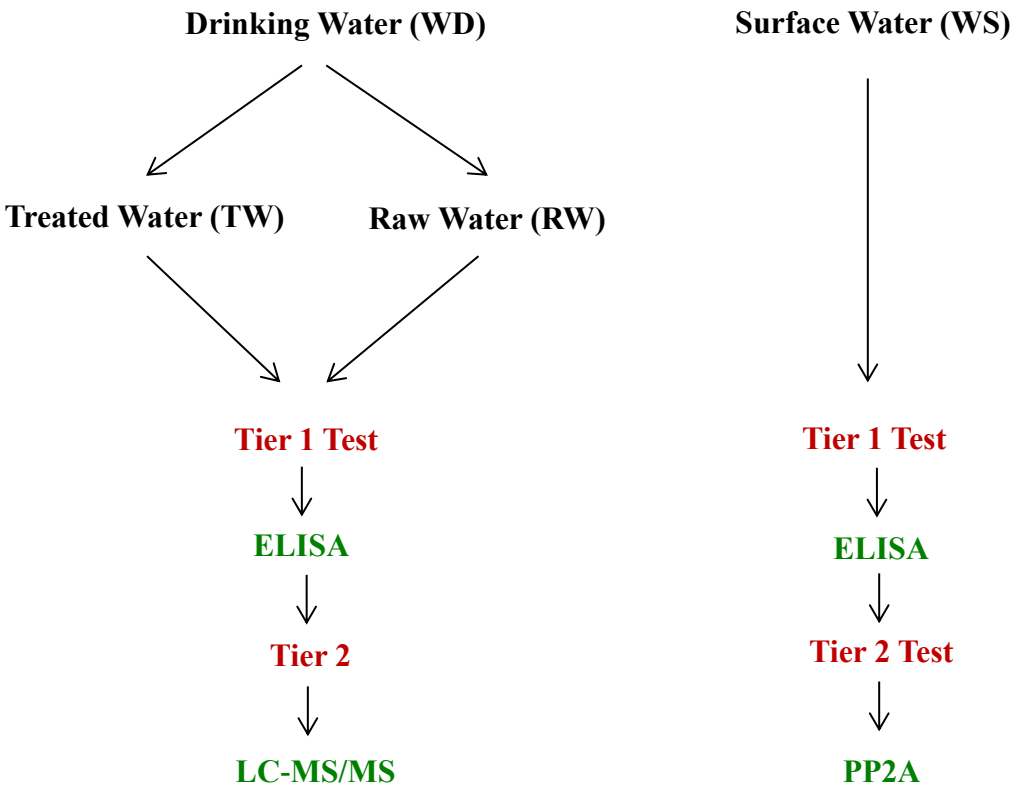

FIGURE 35. TEST ALGROTHEM RECOMMENDED FOR THE ANALYSIS OF MICROCYSTINS

Western blot is another method with the potential to adjudicate discrepant results between ELISA and LC-MS/MS. In a first step, the protein of interest (microcystin) is separated on the basis of their size from a mixture of protein. This separation is achieved by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). In the second step, separated proteins are transferred from the gel onto a nylon membrane by electrophoresis. Third step is blocking the unoccupied binding sites on the membrane. In fourth and fifth step, membrane is incubated with anti-microcystin primary antibodies followed by a second incubation with an appropriate anti-sheep IgG secondary antibody-enzyme conjugate respectively. Finally, the sixth step is the addition of substrate (TMB) which produces color bands on the membrane. These bands are identified according to their location on the membrane by comparing to molecular weight standards. Microcystins are very difficult to detect due to their smaller molecular

weight (1 KD) by using western blot technique. So far no literature is available on a successful western blot for microcystins.

Recovery of microcystins was 50 to 60% after storage of samples from 1-2 years at -16ºC. Hence, to extend the shelf-life of samples, storage at -80ºC or below is recommended.

9 Appendices

APPENDIX A – MICROCYSTINS RESULTS FROM 2010 TO 2012 THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS

TABLE A1. 2010 SURFACE WATER SAMPLES (WS) THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C177701-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
2	C178226-0001	WS	0.1	0.1	0.1	0.1	0.04	0.15
3	C178227-0001	WS	0.25	0.25	0.25	0.25	0.1	0.15
4	C178228-0001	WS	0.1	0.1	0.1	0.1	0.04	0.15
5	C178229-0001	WS	2.5	2.5	2.5	2.5	1.0	0.15
6	C178947-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
7	C179972-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
8	C179982-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
9	C179993-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
10	C180745-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
11	C180745-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
12	C180745-0004	WS	0.05	0.05	0.05	0.05	0.02	0.15
13	C181114-0003	WS	0.05	0.05	0.05	0.05	0.05	0.15
14	C181114-0004	WS	0.05	0.05	0.05	0.05	0.02	0.15
15	C181606-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
16	C181639-0004	WS	0.05	0.05	0.05	0.05	0.02	0.15
17	C181790-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
18	C177206-0001	WS	0.05	0.05	0.05	0.05	0.05	Not Detected
19	C178725-0001	WS	0.1	0.1	0.1	0.1	0.04	Not Detected
20	C178871-0001	WS	0.5	0.5	0.5	0.5	0.2	Not Detected
21	C178947-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
22	C179269-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
23	C179269-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
24	C179269-0004	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
25	C179492-0003	WS	0.05	0.05	0.05	0.05	0.05	Not Detected
26	C179492-0004	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
27	C180576-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
28	C180586-0001	WS	0.5	0.5	0.5	0.5	0.2	Not Detected
29	C181114-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
30	C181114-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
31	C181606-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
32	C181639-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
33	C181639-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
34	C181639-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
35	C181686-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
36	C181686-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
37	C181790-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
38	C181790-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
Total 2010 WS samples = 38								

TABLE A2. 2010 DRINKING WATER SAMPLES (WD) THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS

Counter	Matrix	Sample #	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C177790-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
2	C178303-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
3	C178663-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
4	C178818-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
5	C178890-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
6	C178942-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
7	C179145-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
8	C179430-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
9	C179527-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
10	C179680-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
11	C179718-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
12	C179719-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
13	C179835-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
14	C179857-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
15	C179860-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
16	C180015-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
17	C180043-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
18	C180044-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
19	C180113-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
20	C180263-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
21	C180275-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
22	C180423-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
23	C180488-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
24	C180512-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
25	C180635-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
26	C180637-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
27	C180690-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
28	C180691-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
29	C180833-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
30	C180836-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
31	C180958-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
32	C181079-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
33	C177360-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
34	C177361-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
35	C177362-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
36	C177363-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
37	C177440-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
38	C177478-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
39	C177598-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
40	C177599-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
41	C177600-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
42	C177601-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
43	C177625-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
44	C177626-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
45	C177706-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
46	C177736-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
47	C177786-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
48	C177787-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
49	C177788-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
50	C177791-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
51	C177870-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
52	C177946-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
53	C177987-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
54	C177988-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
55	C178110-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
56	C178142-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
57	C178174-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
58	C178183-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
59	C178249-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
60	C178250-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
61	C178398-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
62	C178425-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
63	C178499-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
64	C178525-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
65	C178526-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
66	C178585-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
67	C178628-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
68	C178735-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
69	C178736-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
70	C178767-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
71	C178817-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
72	C178819-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
73	C179109-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
74	C179214-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
75	C179215-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
76	C179260-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
77	C179323-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
78	C179338-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
79	C179340-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
80	C179502-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
81	C179526-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
82	C179566-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
83	C179591-0002	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
84	C179717-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
85	C179794-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
86	C179957-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
87	C180042-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
88	C180213-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
89	C180260-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
90	C180409-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
91	C180425-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
92	C180426-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
93	C180487-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
94	C180608-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
95	C180634-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
96	C180636-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
97	C180738-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
98	C180834-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
99	C180835-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
100	C180837-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
101	C180895-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
102	C180896-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
103	C180940-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
104	C181048-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
105	C179631-0001	WD	0.1	0.1	0.1	0.1	0.04	Not Detected
Total 2010 WD samples =105								

TABLE A3. 2011 SURFACE WATER SAMPLES (WS) THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C186120-0001	WS	0.07	0.07	0.07	0.07	0.03	0.15
2	C186120-0004	WS	0.1	0.1	0.1	0.1	0.04	0.15
3	C186356-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
4	C187390-0001	WS	0.1	0.1	0.1	0.1	0.04	0.15
5	C187390-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
6	C187404-0002	WS	2.5	2.5	2.5	2.5	1.0	0.15
7	C187405-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
8	C187435-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
9	C187609-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
10	C187609-0002	WS	1.0	1.0	1.0	1.0	0.4	0.15
11	C187628-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
12	C187789-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
13	C187790-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
14	C188297-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
15	C188308-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
16	C188308-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
17	C190314-0003	WS	0.1	0.05	0.05	0.05	0.02	0.15
18	C185590-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
19	C185666-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
20	C185666-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
21	C185700-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
22	C185700-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
23	C186356-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
24	C186430-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
25	C186513-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
26	C186561-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
27	C186561-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
28	C186859-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
29	C187010-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
30	C187124-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
31	C187124-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
32	C187200-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
33	C187200-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
34	C187349-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
35	C187390-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
36	C187435-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
37	C187600-0001	WS	0.1	0.1	0.1	0.1	0.05	Not Detected
38	C187829-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
39	C187847-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
40	C188001-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
41	C188049-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
42	C188063-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
43	C188394-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
44	C189268-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
45	C190197-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
46	C190198-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
47	C190314-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
48	C190314-0004	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
49	C190523-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
50	C185987-0001	WS	0.05	0.12	0.12	0.12	0.05	Not Detected
51	C186172-0001	WS	0.12	0.12	0.12	0.12	0.05	Not Detected
52	C186620-0001	WS	0.08	0.08	0.08	0.08	0.03	Not Detected
53	C186620-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
54	C187349-0002	WS	0.1	0.1	0.1	0.1	0.04	Not Detected
55	C188696-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
56	C188938-0001	WS	0.05	0.05	0.05	0.07	0.02	Not Detected
Total 2011 WS samples = 56								

TABLE A4. 2011 DRINKING WATER SAMPLES (WD) THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS BOTH

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C186067-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
2	C186069-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
3	C186125-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
4	C186303-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
5	C186304-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
6	C186305-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
7	C187409-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
8	C187741-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
9	C187742-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
10	C187884-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
11	C188171-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
12	C188293-0003	WD	0.05	0.05	0.05	0.05	0.02	0.15
13	C188684-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
14	C189794-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
15	C190234-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
16	C185820-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
17	C185821-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
18	C185896-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
19	C185934-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
20	C186126-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
21	C186262-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
22	C186477-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
23	C186533-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
24	C186606-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
25	C186656-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
26	C187146-0001	WD	0.05	0.05	0.09	0.05	0.02	Not Detected
27	C187281-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
28	C187389-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
29	C187468-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
30	C187558-0001	WD	0.05	0.05	0.19	0.05	0.02	Not Detected
31	C187559-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
32	C187630-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
33	C187666-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
34	C187841-0002	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
35	C188129-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
36	C188373-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
37	C188388-0001	WD	0.05	0.05	0.05	0.07	0.02	Not Detected
38	C188388-0002	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
39	C188438-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
40	C188766-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
41	C188907-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
42	C188991-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
43	C189039-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
44	C189304-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
45	C189305-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
46	C189742-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
47	C189894-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
48	C189970-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
49	C189971-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
50	C190025-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
51	C190026-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
52	C190027-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
53	C190071-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
54	C190072-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
55	C190207-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
56	C190209-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
57	C190210-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
58	C190236-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
59	C190237-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
60	C190291-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
61	C190292-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
62	C190408-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
63	C190409-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
64	C190412-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
65	C190413-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
66	C190445-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
67	C190446-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
68	C190448-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
69	C190579-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
70	C190580-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
71	C190582-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
72	C190583-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
73	C190584-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
74	C190607-0003	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
75	C190609-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
76	C190761-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
77	C190762-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
78	C190763-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
79	C190798-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
80	C190799-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
81	C190800-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
82	C190881-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
83	C191139-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
Total 2011 WD samples = 83								

TABLE A5 2012 SURFACE WATER SAMPLES (WS) THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS BOTH

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C195037-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
2	C195159-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
3	C195179-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
4	C195240-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
5	C195448-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
6	C195580-0001	WS	0.05	0.05	0.05	0.05	0.04	0.15
7	C195637-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
8	C195637-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
9	C195677-0001	WS	0.05	0.05	0.05	0.05	1.0	0.15
10	C195979-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
11	C196032-0001	WS	0.05	0.05	0.05	0.05	0.03	0.15
12	C196195-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
13	C196196-0001	WS	0.05	0.05	0.05	0.05	0.11	0.15
14	C196200-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
15	C196200-0002	WS	0.05	0.05	0.05	0.05	0.02	0.15
16	C196605-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
17	C196956-0001	WS	0.05	0.05	0.05	0.05	0.02	0.15
18	C198562-0001	WS	0.07	0.05	0.05	0.05	0.02	0.15
19	C196414-0001	WS	0.1	0.1	0.1	0.09	0.04	0.15
20	C196592-0001	WS	0.11	0.05	0.05	0.09	0.02	0.15
21	C193172-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
22	C194521-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
23	C194907-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
24	C194923-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
25	C195240-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
26	C195486-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
27	C195486-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
28	C195726-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
29	C195859-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
30	C195979-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
31	C196032-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
32	C196332-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
33	C196353-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
34	C196413-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
35	C196413-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
36	C196413-0004	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
37	C196527-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
38	C196527-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
39	C196586-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
40	C196586-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
41	C196586-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
42	C196997-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
43	C197008-0012	WS	0.05	0.05	0.05	0.05	0.03	Not Detected
44	C197190-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
45	C197190-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
46	C197190-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
47	C197228-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
48	C197228-0002	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
49	C197228-0003	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
50	C197228-0004	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
51	C197375-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
52	C197826-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
53	C198407-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
54	C198934-0001	WS	0.05	0.05	0.05	0.05	0.02	Not Detected
55	C196752-0003	WS	0.1	0.1	0.1	0.1	0.04	Not Detected
Total 2012WS samples = 55								

TABLE A6. 2012 DRINKING WATER (WD) SAMPLES THAT WERE NEGATIVE BY BOTH ELISA AND LC-MS/MS

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C191512-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
2	C191643-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
3	C191888-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
4	C192116-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
5	C192364-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
6	C192647-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
7	C192912-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
8	C193110-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
9	C193451-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
10	C193785-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
11	C194131-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
12	C194508-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
13	C194674-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
14	C194676-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
15	C194677-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
16	C194886-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
17	C194889-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
18	C196403-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
19	C196404-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
20	C196409-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
21	C196617-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
22	C196652-0003	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
23	C196945-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
24	C196946-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
25	C197147-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
26	C197148-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
27	C197330-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
28	C197693-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
29	C197882-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
30	C197885-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
31	C197886-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
32	C197887-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
33	C197993-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
34	C198071-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
35	C198072-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
36	C198073-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
37	C198074-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
38	C198127-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
39	C198267-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
40	C198325-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
41	C198331-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
42	C198445-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
43	C198544-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
44	C198547-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
45	C198549-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
46	C198596-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
47	C198736-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
48	C198739-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
49	C198807-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
50	C198955-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
51	C198991-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
52	C199037-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
53	C199067-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
54	C199136-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
55	C199137-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
56	C199139-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
57	C199140-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
58	C199348-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
59	C199354-0001	WD	0.05	0.05	0.05	0.05	0.02	Not Detected
60	C197484-0001	WD	0.05	0.05	0.05	0.05	0.03	Not Detected
61	C194719-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
62	C194884-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
63	C195087-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
64	C195127-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
65	C196239-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
66	C196240-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
67	C196242-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
68	C196245-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
69	C196334-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
70	C196402-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
71	C196408-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
72	C196495-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
73	C196652-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
74	C196652-0002	WD	0.05	0.05	0.05	0.05	0.02	0.15
75	C196730-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
76	C196767-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
77	C196768-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
78	C196770-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
79	C196773-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
80	C196980-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
81	C197053-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
82	C197142-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
83	C197144-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
84	C197368-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
85	C197483-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
86	C197485-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
87	C197648-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
88	C197692-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
89	C197694-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
90	C197695-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
91	C197779-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
92	C197820-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
93	C197881-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
94	C197883-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
95	C198126-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
96	C198326-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
97	C198738-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
98	C198806-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
99	C198951-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
100	C198952-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
101	C198954-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
102	C199135-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
103	C199349-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
104	C199350-0001	WD	0.05	0.05	0.05	0.05	0.02	0.15
105	C194887-0001	WD	0.06	0.05	0.05	0.05	0.02	0.15
106	C196400-0001	WD	0.05	0.05	0.06	0.05	0.02	0.15
107	C196610-0001	WD	0.05	0.05	0.08	0.05	0.02	0.15
108	C197819-0001	WD	0.08	0.05	0.12	0.05	0.02	0.15
109	C197329-0001	WD	0.07	0.05	0.05	0.06	0.02	0.15
110	C196543-0001	WD	0.05	0.05	0.05	0.07	0.02	0.15
111	C196731-0001	WD	0.05	0.05	0.05	0.07	0.02	0.15
112	C197442-0001	WD	0.08	0.05	0.05	0.09	0.02	0.15
113	C197327-0001	WD	0.1	0.05	0.05	0.12	0.02	0.15
Total 2012WD samples = 113								

APPENDIX B - GRAPHICAL PRESENTATION OF AGREEMENT CALCULATION BETWEEN ELISA AND LC-MS/MS

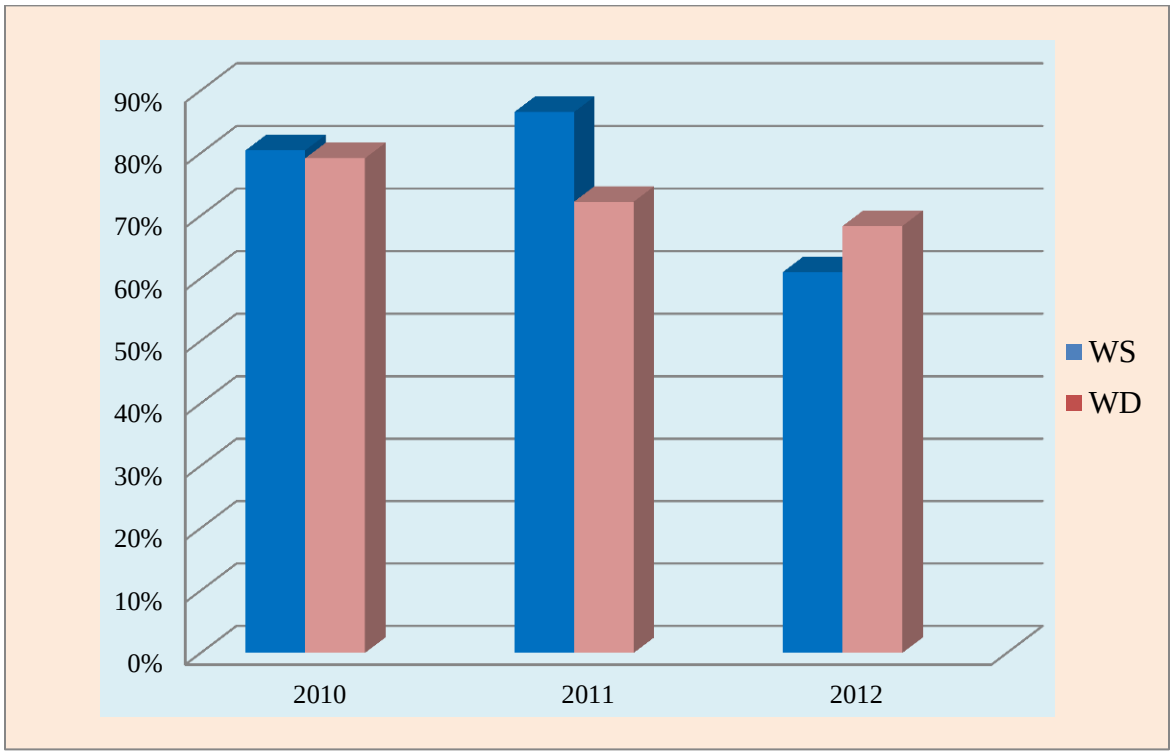

FIGURE B1. 2010 TO 2012 (WS & WD) MICROCYSTINS RESULTS AGREEMENT BETWEEN ELISA & LC-MS/MS

APPENDIX C – MICROCYSTINS RESULTS FROM LC-MS/MS WITH HIGH MATRIX INTERFERENCE

TABLE C1.LC-MS/MS MICROCYSTINS TEST RESULTS (2010-2012) OF SURFACE WATER SAMPLES WITH HIGH MATRIX INTERFERENCE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C182002-0001	WS	0.5	0.5	0.5	0.5	0.2	0.41
2	C178947-0001	WS	1.0	2.0	2.0	2.0	0.5	2.8
3	C179406-0001	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
4	C181378-0001	WS	1.5	0.25	0.25	0.76	0.1	0.5
5	C179269-0001	WS	0.49	0.83	0.83	0.83	0.42	1.2
6	C178226-0001	WS	0.1	0.1	0.1	0.1	0.04	0.15
7	C178227-0001	WS	0.25	0.25	0.25	0.25	0.1	0.15
8	C178228-0001	WS	0.1	0.1	0.1	0.1	0.04	0.15
9	C178229-0001	WS	2.5	2.5	2.5	2.5	1.0	0.15
10	C178725-0001	WS	0.1	0.1	0.1	0.1	0.04	Not Detected
11	C178871-0001	WS	0.5	0.5	0.5	0.5	0.2	Not Detected
12	C180586-0001	WS	0.5	0.5	0.5	0.5	0.2	Not Detected
13	C186289-0001	WS	0.83	0.83	0.83	0.83	0.33	0.2
14	C187404-0001	WS	1.2	1.2	1.2	1.2	0.5	0.4
15	C187406-0002	WS	0.5	0.5	0.5	0.5	0.2	2.3
16	C187565-0001	WS	2.5	2.5	2.5	2.5	1.0	32.7
17	C189074-0001	WS	0.35	0.5	0.5	0.5	0.2	0.15
18	C186289-0002	WS	0.44	0.25	0.25	0.25	0.1	Not Detected
19	C188890-0001	WS	0.19	0.5	0.5	0.13	0.2	Not Detected
20	C188064-0001	WS	1.1	0.5	0.5	0.96	0.2	4.5
21	C187406-0001	WS	0.8	0.5	0.5	0.7	0.2	6.4
22	C188326-0002	WS	2.4	2.5	3.5	2.5	1.0	13.05
23	C189234-0001	WS	2.5	2.5	5.7	2.5	1.0	50
24	C186621-0001	WS	12	0.12	0.12	12	0.05	63.9
25	C187436-0001	WS	6.6	3.4	1.0	1.0	0.5	95.6
26	C185928-0001	WS	28	5	5	5.1	2	126.5
27	C188252-0004	WS	30	14	2.4	2.4	0.02	170
28	C188937-0001	WS	0.5	0.5	0.5	0.5	3000	0.55
29	C188889-0001	WS	340	5.0	5.0	2000	2.0	700
30	C186120-0003	WS	0.13	0.25	0.25	0.24	0.1	1.5
31	C186120-0001	WS	0.07	0.07	0.07	0.07	0.03	0.15
32	C186120-0004	WS	0.1	0.1	0.1	0.1	0.04	0.15

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
33	C187390-0001	WS	0.1	0.1	0.1	0.1	0.04	0.15
34	C187404-0002	WS	2.5	2.5	2.5	2.5	1.0	0.15
35	C187609-0002	WS	1.0	1.0	1.0	1.0	0.4	0.15
36	C187600-0001	WS	0.1	0.1	0.1	0.1	0.05	Not Detected
37	C185987-0001	WS	0.05	0.12	0.12	0.12	0.05	Not Detected
38	C186172-0001	WS	0.12	0.12	0.12	0.12	0.05	Not Detected
39	C186620-0001	WS	0.08	0.08	0.08	0.08	0.03	Not Detected
40	C187349-0002	WS	0.1	0.1	0.1	0.1	0.04	Not Detected
41	C196592-0002	WS	0.12	0.05	0.05	0.12	0.02	0.3
42	C196752-0001	WS	0.12	0.06	0.12	0.12	0.05	0.45
43	C196641-0001	WS	0.06	0.12	0.12	0.12	0.05	1.3
44	C196872-0001	WS	0.08	0.07	0.08	0.08	0.03	2.25
45	C196587-0001	WS	0.12	0.05	0.12	0.09	0.05	2.45
46	C196124-0001	WS	0.05	0.25	0.06	0.25	0.1	Not Detected
47	C193446-0001	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
48	C194522-0001	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
49	C196026-0002	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
50	C196149-0003	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
51	C196149-0004	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
52	C196752-0004	WS	0.25	0.25	0.25	0.25	0.1	Not Detected
53	C193850-0006	WS	0.5	0.5	0.5	0.5	0.2	Not Detected
54	C196458-0001	WS	0.5	0.5	0.5	0.5	0.2	Not Detected
55	C196026-0001	WS	1.3	1.3	1.3	1.3	0.5	Not Detected
56	C197894-0001	WS	5.0	5.0	5.0	5.0	2.0	Not Detected
57	C196124-0002	WS	0.25	0.25	0.21	0.25	0.1	0.15
58	C196130-0001	WS	0.25	0.25	0.25	0.25	0.1	0.15
59	C196532-0001	WS	0.25	0.25	0.25	0.25	0.1	0.15
60	C196997-0001	WS	0.25	0.25	0.25	0.25	0.1	0.15
61	C196588-0001	WS	0.25	0.25	0.25	0.08	0.1	0.45
62	C196897-0001	WS	0.22	0.08	0.08	0.08	0.03	0.85
63	C196587-0003	WS	0.25	0.25	0.25	0.39	0.1	2.85
64	C196027-0001	WS	0.52	0.12	0.44	0.52	0.05	2.95
65	C196275-0001	WS	0.5	0.5	0.5	0.5	0.03	3.5
66	C196944-0001	WS	0.12	3.2	0.12	0.12	0.05	13.2
67	C196998-0001	WS	0.25	0.08	0.08	0.08	0.03	1.2

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
68	C196752-0002	WS	0.12	0.15	0.12	0.12	0.05	2.15
69	C197056-0001	WS	0.23	0.25	0.25	0.1	0.1	2.6
70	C196897-0002	WS	0.5	0.5	0.5	0.5	0.2	1.2
71	C197220-0001	WS	1.4	0.5	0.5	0.34	0.13	1.55
72	C196532-0002	WS	0.5	0.5	0.5	0.5	0.2	0.25
73	C196274-0001	WS	0.5	0.5	0.5	0.5	0.2	0.45
74	C196414-0001	WS	0.1	0.1	0.1	0.09	0.04	0.15
75	C196752-0003	WS	0.1	0.1	0.1	0.1	0.04	Not Detected
LC-MS/MS matrix interference in WS samples = 75/854*100 =8.8%								

TABLEC 2. LC-MS/MS MICROCYSTINS RESULTS (2010 TO 2012) OF DRINKING WATER SAMPLES WITH HIGH MATRIX INTERFERENCE

Counter	Sample #	Matrix	Microcystins Variants (µg/L)				Anatoxin-a (µg/L)	ELISA (µg/L)
			-LR	-YR	-RR	-LA		
1	C180262-0001	WD	0.06	0.06	0.05	0.05	0.02	Not Detected
2	C178500-0001	WD	0.08	0.06	0.05	0.06	0.02	0.2
3	C179386-0001	WD	0.07	0.06	0.05	0.07	0.02	0.3
4	C180041-0001	WD	0.06	0.09	0.05	0.06	0.02	0.35
5	C179339-0001	WD	0.08	0.08	0.05	0.06	0.02	0.4
6	C179631-0001	WD	0.1	0.1	0.1	0.1	0.04	Not Detected
7	C187466-0001	WD	0.07	0.07	0.05	0.05	0.02	0.15
8	C188908-0001	WD	0.1	0.05	0.05	0.1	0.02	0.15
9	C187496-0001	WD	0.06	0.06	0.05	0.05	0.02	0.4
10	C188130-0001	WD	0.09	0.09	0.05	0.05	0.02	0.4
11	C188295-0002	WD	0.06	0.06	0.05	0.05	0.02	0.6
12	C186713-0001	WD	0.13	0.08	0.05	0.08	0.02	0.9
13	C187004-0001	WD	0.09	0.09	0.05	0.05	0.02	1.15
14	C195613-0001	WD	0.13	0.05	0.13	0.05	0.02	0.35
15	C197479-0001	WD	0.15	0.05	0.15	0.06	0.17	0.75
LC-MS/MS matrix interference in WD = 15/854*100 =1.8%								

APPENDIX D -WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

TABLE D1. 2010 WS WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2010 WS Samples	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	77	40	117
	No	0		1
		77		117
Percentage of microcystins workload relieved by ELISA				**34%**
LC-MS/MS contribution to total workload				**66%**

TABLE D2. 2010 WD WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2010 WD Samples	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	148	206	354
	No	13		13
		161		367
Percentage of microcystins workload relieved by ELISA				**56%**
LC-MS/MS contribution to total workload				**44%**

TABLE D3. 2010 (WS & WD) WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2010 WS &WD Samples	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	225	246	471
	No	13		13
		238		484
Percentage of microcystins workload relieved by ELISA				**51%**
LC-MS/MS contribution to total workload				**49%**

TABLE D4. 2011 WS WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2011WS Samples		LC-MS/MS Tested		Total
		Yes	No	
ELISA Tested	Yes	104	7	111
	No	0		0
		104		111
Percentage of microcystins workload relieved by ELISA				6%
LC-MS/MS contribution to total workload				94%

TABLE D5. 2011 WD WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2011 WD Samples		LC-MS/MS Tested		Total
		Yes	No	
ELISA Tested	Yes	172	335	507
	No	10		10
		182		517
Percentage of microcystins workload relieved by ELISA				68%
LC-MS/MS contribution to total workload				32%

TABLE D6. 2011 (WS & WD) WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2011 WS & WD Samples		LC-MS/MS Tested		Total
		Yes	No	
ELISA Tested	Yes	276	342	618
	No	10		10
		286		628
Percentage of microcystins workload relieved by ELISA				54%
LC-MS/MS contribution to total workload				46%

TABLE D7. 2012 WS WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2012 WS Samples		LC-MS/MS Tested		Total
		Yes	No	
ELISA Tested	Yes	134	59	193
	No	11		11
		145		204
Percentage of microcystins workload relieved by ELISA				29%
LC-MS/MS contribution to total workload				71%

TABLE D8. 2012 WD WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2012 WD Samples		LC-MS/MS Tested		Total
		Yes	No	
ELISA Tested	Yes	219	423	642
	No	12		12
		231		654
Percentage of microcystins workload relieved by ELISA				65%
LC-MS/MS contribution to total workload				35%

TABLE D9. 2012 (WS & WD) WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2012 WS & WD Samples		LC-MS/MS Tested		Total
		Yes	No	
ELISA Tested	Yes	353	482	835
	No	23		23
		376		858
Percentage of microcystins workload relieved by ELISA				56%
LC-MS/MS contribution to total workload				44%

TABLE D10. 2010 TO 2012 (WS & WD) WORKLOAD CONTRIBUTION OF ELISA AND LC-MS/MS

2010 to 2012 (WS & WD) Samples	**LC-MS/MS Tested**			**Total**
		Yes	**No**	
ELISA Tested	**Yes**	854	1070	1924
	No	46		45
		900		1970
Percentage of microcystins workload relieved by ELISA				**54%**
LC-MS/MS contribution to total workload				**46%**

APPENDIX E – MICROCYSTINS RESULTS SORTED ON THE BASIS OF DETECTION METHOD (ELISA OR LC-MS/MS OR BY BOTH)

TABLE E1. 2010 SURFACE WATER (WS) SAMPLES TESTED BY ONLY ELISA FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	31-May-10	C176724-0001	15	19-Aug-10	C179296-0002	29	07-Sep-10	C179693-0001
2	03-Jun-10	C176884-0001	16	19-Aug-10	C179296-0003	30	10-Sep-10	C179812-0001
3	07-Jun-10	C176928-0001	17	19-Aug-10	C179296-0004	31	10-Sep-10	C179812-0002
4	22-Jun-10	C177385-0001	18	23-Aug-10	C179325-0001	32	14-Sep-10	C179862-0001
5	23-Jun-10	C177460-0001	19	31-Aug-10	C179523-0001	33	21-Sep-10	C180054-0001
6	30-Jun-10	C177641-0001	20	31-Aug-10	C179523-0002	34	23-Sep-10	C180124-0001
7	12-Jul-10	C177962-0001	21	01-Sep-10	C179574-0001	35	23-Sep-10	C180124-0002
8	28-Jul-10	C178505-0001	22	01-Sep-10	C179576-0001	36	30-Sep-10	C180343-0002
9	30-Jul-10	C178581-0001	23	01-Sep-10	C179577-0001	37	30-Sep-10	C180343-0003
10	05-Aug-10	C178680-0001	24	01-Sep-10	C179588-0001	38	30-Sep-10	C180343-0004
11	12-Aug-10	C178944-0001	25	01-Sep-10	C179588-0002	39	16-Nov-10	C181624-0001
12	18-Aug-10	C179246-0001	26	03-Sep-10	C179635-0001	40	16-Nov-10	C181624-0002
13	19-Aug-10	C179276-0001	27	07-Sep-10	C179686-0001			
14	19-Aug-10	C179296-0001	28	07-Sep-10	C179687-0001			
2010 WS samples tested by ELISA only = 40								

TABLE E2. 2010 SURFACE WATER (WS) SAMPLES TESTED BY LC-MS/MS ONLY FOR MICROCYSTINS

Counter	Date	Sample I.D
2010 WS samples tested by LC-MS/MS only = 0		

TABLE E3. 2010 SURFACE WATER (WS) SAMPLES TESTED BY BOTH ELISA AND LC-MS/MS

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
1	16-Jun-10	C177206-0001	25	25-Aug-10	C179406-0001	49	30-Sep-10	C180343-0001
2	16-Jun-10	C177206-0002	26	27-Aug-10	C179492-0001	50	07-Oct-10	C180541-0001
3	02-Jul-10	C177701-0001	27	27-Aug-10	C179492-0002	51	08-Oct-10	C180576-0001
4	02-Jul-10	C177701-0002	28	27-Aug-10	C179492-0003	52	08-Oct-10	C180586-0001
5	20-Jul-10	C178226-0001	29	27-Aug-10	C179492-0004	53	15-Oct-10	C180745-0001
6	20-Jul-10	C178227-0001	30	27-Aug-10	C179492-0005	54	15-Oct-10	C180745-0002
7	20-Jul-10	C178228-0001	31	27-Aug-10	C179492-0006	55	15-Oct-10	C180745-0003
8	20-Jul-10	C178229-0001	32	31-Aug-10	C179540-0001	56	15-Oct-10	C180745-0004
9	06-Aug-10	C178725-0001	33	31-Aug-10	C179540-0002	57	20-Oct-10	C180932-0001
10	11-Aug-10	C178871-0001	34	01-Sep-10	C179589-0001	58	27-Oct-10	C181114-0001
11	11-Aug-10	C178878-0001	35	16-Sep-10	C179958-0001	59	27-Oct-10	C181114-0002
12	12-Aug-10	C178947-0001	36	16-Sep-10	C179958-0002	60	27-Oct-10	C181114-0003
13	12-Aug-10	C178947-0002	37	16-Sep-10	C179972-0001	61	27-Oct-10	C181114-0004
14	12-Aug-10	C178947-0003	38	16-Sep-10	C179982-0001	62	04-Nov-10	C181378-0001
15	12-Aug-10	C178947-0004	39	16-Sep-10	C179982-0002	63	16-Nov-10	C181606-0001
16	13-Aug-10	C179088-0001	40	16-Sep-10	C179982-0003	64	16-Nov-10	C181606-0002
17	13-Aug-10	C179089-0001	41	16-Sep-10	C179982-0004	65	17-Nov-10	C181639-0001
18	19-Aug-10	C179269-0001	42	17-Sep-10	C179993-0001	66	17-Nov-10	C181639-0002
19	19-Aug-10	C179269-0002	43	23-Sep-10	C180126-0001	67	17-Nov-10	C181639-0003
20	19-Aug-10	C179269-0003	44	28-Sep-10	C180253-0001	68	17-Nov-10	C181639-0004
21	19-Aug-10	C179269-0004	45	28-Sep-10	C180253-0002	69	17-Nov-10	C181639-0005
22	23-Aug-10	C179324-0001	46	29-Sep-10	C180295-0001	70	17-Nov-10	C181639-0006
23	24-Aug-10	C179357-0001	47	30-Sep-10	C180341-0001	71	17-Nov-10	C181639-0007
24	24-Aug-10	C179357-0002	48	30-Sep-10	C180341-0002	72	18-Nov-10	C181686-0001

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	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
73	18-Nov-10	C181686-0002	75	23-Nov-10	C181790-0002	77	01-Dec-10	C182002-0001
74	23-Nov-10	C181790-0001	76	23-Nov-10	C181790-0003			
2010 WS samples tested by both ELISA and LC-MS/MS = 77								

TABLE E4. 2010 DRINKING WATER (WD) SAMPLES TESTED BY ELISA ONLY FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	03-Mar-10	C174890-0001	28	22-Jun-10	C177363-0002	55	15-Jul-10	C178110-0002
2	03-Mar-10	C174890-0002	29	23-Jun-10	C177440-0002	56	16-Jul-10	C178142-0002
3	03-Mar-10	C174890-0003	30	23-Jun-10	C177441-0002	57	19-Jul-10	C178174-0002
4	03-Mar-10	C174890-0004	31	24-Jun-10	C177478-0002	58	20-Jul-10	C178182-0002
5	03-Mar-10	C174890-0005	32	29-Jun-10	C177598-0002	59	20-Jul-10	C178183-0002
6	03-Mar-10	C174890-0006	33	29-Jun-10	C177599-0002	60	21-Jul-10	C178243-0002
7	03-Mar-10	C174890-0007	34	29-Jun-10	C177600-0002	61	21-Jul-10	C178249-0002
8	03-Mar-10	C174890-0008	35	29-Jun-10	C177601-0002	62	21-Jul-10	C178250-0002
9	03-Mar-10	C174890-0009	36	30-Jun-10	C177625-0002	63	21-Jul-10	C178270-0002
10	03-Mar-10	C174890-0010	37	30-Jun-10	C177626-0002	64	21-Jul-10	C178271-0002
11	03-Mar-10	C174890-0011	38	30-Jun-10	C177627-0002	65	22-Jul-10	C178303-0002
12	03-Mar-10	C174890-0012	39	02-Jul-10	C177706-0002	66	26-Jul-10	C178398-0002
13	15-Jun-10	C177122-0001	40	06-Jul-10	C177736-0002	67	27-Jul-10	C178423-0002
14	15-Jun-10	C177122-0002	41	07-Jul-10	C177786-0002	68	27-Jul-10	C178424-0002
15	15-Jun-10	C177123-0001	42	07-Jul-10	C177787-0002	69	27-Jul-10	C178425-0002
16	15-Jun-10	C177123-0002	43	07-Jul-10	C177788-0002	70	28-Jul-10	C178499-0002
17	15-Jun-10	C177124-0001	44	07-Jul-10	C177789-0002	71	28-Jul-10	C178500-0002
18	15-Jun-10	C177124-0002	45	07-Jul-10	C177790-0002	72	29-Jul-10	C178525-0002
19	15-Jun-10	C177125-0001	46	07-Jul-10	C177791-0002	73	29-Jul-10	C178526-0002
20	15-Jun-10	C177125-0002	47	08-Jul-10	C177870-0002	74	03-Aug-10	C178585-0002
21	16-Jun-10	C177182-0002	48	12-Jul-10	C177946-0002	75	03-Aug-10	C178592-0001
22	17-Jun-10	C177268-0002	49	13-Jul-10	C177986-0002	76	03-Aug-10	C178592-0002
23	18-Jun-10	C177309-0002	50	13-Jul-10	C177987-0002	77	03-Aug-10	C178592-0003
24	18-Jun-10	C177310-0002	51	13-Jul-10	C177988-0002	78	04-Aug-10	C178626-0002
25	22-Jun-10	C177360-0002	52	14-Jul-10	C178063-0002	79	04-Aug-10	C178627-0002
26	22-Jun-10	C177361-0002	53	14-Jul-10	C178064-0001	80	04-Aug-10	C178628-0002
27	22-Jun-10	C177362-0002	54	14-Jul-10	C178064-0002	81	05-Aug-10	C178663-0002

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Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
82	05-Aug-10	C178664-0002	109	17-Aug-10	C179126-0002	136	31-Aug-10	C179526-0002
83	06-Aug-10	C178735-0002	110	17-Aug-10	C179143-0002	137	31-Aug-10	C179527-0002
84	06-Aug-10	C178736-0002	111	17-Aug-10	C179144-0002	138	01-Sep-10	C179566-0002
85	09-Aug-10	C178767-0002	112	17-Aug-10	C179145-0002	139	01-Sep-10	C179567-0002
86	10-Aug-10	C178817-0002	113	18-Aug-10	C179214-0002	140	01-Sep-10	C179591-0001
87	10-Aug-10	C178818-0002	114	18-Aug-10	C179215-0002	141	03-Sep-10	C179636-0002
88	10-Aug-10	C178819-0002	115	19-Aug-10	C179260-0002	142	07-Sep-10	C179680-0002
89	10-Aug-10	C178820-0002	116	19-Aug-10	C179261-0002	143	08-Sep-10	C179716-0002
90	11-Aug-10	C178889-0002	117	19-Aug-10	C179272-0001	144	08-Sep-10	C179717-0002
91	11-Aug-10	C178890-0002	118	19-Aug-10	C179272-0002	145	08-Sep-10	C179718-0002
92	12-Aug-10	C178934-0001	119	19-Aug-10	C179273-0001	146	08-Sep-10	C179719-0002
93	12-Aug-10	C178934-0002	120	19-Aug-10	C179273-0002	147	09-Sep-10	C179740-0002
94	12-Aug-10	C178935-0001	121	19-Aug-10	C179274-0001	148	10-Sep-10	C179794-0002
95	12-Aug-10	C178935-0002	122	19-Aug-10	C179274-0002	149	13-Sep-10	C179834-0002
96	12-Aug-10	C178936-0001	123	23-Aug-10	C179323-0002	150	13-Sep-10	C179835-0002
97	12-Aug-10	C178936-0002	124	24-Aug-10	C179337-0002	151	14-Sep-10	C179857-0002
98	12-Aug-10	C178936-0003	125	24-Aug-10	C179338-0002	152	14-Sep-10	C179858-0002
99	12-Aug-10	C178936-0004	126	24-Aug-10	C179339-0002	153	14-Sep-10	C179859-0002
100	12-Aug-10	C178942-0002	127	24-Aug-10	C179340-0002	154	14-Sep-10	C179860-0002
101	13-Aug-10	C179090-0001	128	25-Aug-10	C179386-0002	155	15-Sep-10	C179907-0002
102	13-Aug-10	C179090-0002	129	26-Aug-10	C179429-0002	156	16-Sep-10	C179956-0002
103	13-Aug-10	C179091-0001	130	26-Aug-10	C179430-0002	157	16-Sep-10	C179957-0002
104	13-Aug-10	C179091-0002	131	30-Aug-10	C179502-0002	158	17-Sep-10	C179990-0001
105	16-Aug-10	C179109-0002	132	31-Aug-10	C179522-0001	159	17-Sep-10	C179990-0002
106	17-Aug-10	C179125-0001	133	31-Aug-10	C179522-0002	160	17-Sep-10	C179991-0001
107	17-Aug-10	C179125-0002	134	31-Aug-10	C179524-0002	161	17-Sep-10	C179991-0002
108	17-Aug-10	C179126-0001	135	31-Aug-10	C179525-0002	162	17-Sep-10	C179992-0001

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
163	17-Sep-10	C179992-0002	178	29-Sep-10	C180276-0002	193	14-Oct-10	C180690-0002
164	20-Sep-10	C180015-0002	179	30-Sep-10	C180357-0002	194	14-Oct-10	C180691-0002
165	21-Sep-10	C180041-0002	180	04-Oct-10	C180409-0002	195	15-Oct-10	C180738-0002
166	21-Sep-10	C180042-0002	181	05-Oct-10	C180423-0002	196	19-Oct-10	C180833-0002
167	21-Sep-10	C180043-0002	182	05-Oct-10	C180424-0002	197	19-Oct-10	C180834-0002
168	21-Sep-10	C180044-0002	183	05-Oct-10	C180425-0002	198	19-Oct-10	C180835-0002
169	22-Sep-10	C180071-0002	184	05-Oct-10	C180426-0002	199	19-Oct-10	C180836-0002
170	23-Sep-10	C180113-0002	185	06-Oct-10	C180487-0002	200	19-Oct-10	C180837-0002
171	23-Sep-10	C180114-0002	186	06-Oct-10	C180488-0002	201	20-Oct-10	C180895-0002
172	27-Sep-10	C180213-0002	187	07-Oct-10	C180512-0002	202	20-Oct-10	C180896-0002
173	28-Sep-10	C180260-0002	188	12-Oct-10	C180608-0002	203	20-Oct-10	C180940-0002
174	28-Sep-10	C180261-0002	189	13-Oct-10	C180634-0002	204	21-Oct-10	C180958-0002
175	28-Sep-10	C180262-0002	190	13-Oct-10	C180635-0002	205	25-Oct-10	C181048-0002
176	28-Sep-10	C180263-0002	191	13-Oct-10	C180636-0002	206	26-Oct-10	C181079-0002
177	29-Sep-10	C180275-0002	192	13-Oct-10	C180637-0002			
Total 2010 WD samples tested by ELISA only = 206								

TABLE E5. 2010 DRINKING WATER (WD) SAMPLES TESTED LC-MS/MS ONLY FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	26-Apr-10	C175910-0001	6	26-Apr-10	C175913-0003	11	04-Aug-10	C178629-0001
2	26-Apr-10	C175910-0002	7	16-Jun-10	C177182-0001	12	04-Aug-10	C178629-0002
3	26-Apr-10	C175910-0003	8	17-Jun-10	C177268-0001	13	04-Aug-10	C178629-0003
4	26-Apr-10	C175913-0001	9	18-Jun-10	C177309-0001			
5	26-Apr-10	C175913-0002	10	18-Jun-10	C177310-0001			
Total 2010 WD samples tested by only LC-MS/MS = 13								

TABLE E6. 2010 DRINKING WATER (WD) SAMPLES TESTED BY BOTH ELISA AND LC-MS/MS

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
1	22-Jun-10	C177360-0001	25	13-Jul-10	C177986-0001	49	04-Aug-10	C178627-0001
2	22-Jun-10	C177361-0001	26	13-Jul-10	C177987-0001	50	04-Aug-10	C178628-0001
3	22-Jun-10	C177362-0001	27	13-Jul-10	C177988-0001	51	05-Aug-10	C178663-0001
4	22-Jun-10	C177363-0001	28	14-Jul-10	C178063-0001	52	05-Aug-10	C178664-0001
5	23-Jun-10	C177440-0001	29	15-Jul-10	C178110-0001	53	06-Aug-10	C178735-0001
6	23-Jun-10	C177441-0001	30	16-Jul-10	C178142-0001	54	06-Aug-10	C178736-0001
7	24-Jun-10	C177478-0001	31	19-Jul-10	C178174-0001	55	09-Aug-10	C178767-0001
8	29-Jun-10	C177598-0001	32	20-Jul-10	C178182-0001	56	10-Aug-10	C178817-0001
9	29-Jun-10	C177599-0001	33	20-Jul-10	C178183-0001	57	10-Aug-10	C178818-0001
10	29-Jun-10	C177600-0001	34	21-Jul-10	C178249-0001	58	10-Aug-10	C178819-0001
11	29-Jun-10	C177601-0001	35	21-Jul-10	C178250-0001	59	10-Aug-10	C178820-0001
12	30-Jun-10	C177625-0001	36	21-Jul-10	C178270-0001	60	11-Aug-10	C178889-0001
13	30-Jun-10	C177626-0001	37	21-Jul-10	C178271-0001	61	11-Aug-10	C178890-0001
14	30-Jun-10	C177627-0001	38	22-Jul-10	C178303-0001	62	12-Aug-10	C178942-0001
15	02-Jul-10	C177706-0001	39	26-Jul-10	C178398-0001	63	16-Aug-10	C179109-0001
16	06-Jul-10	C177736-0001	40	27-Jul-10	C178423-0001	64	17-Aug-10	C179143-0001
17	07-Jul-10	C177786-0001	41	27-Jul-10	C178424-0001	65	17-Aug-10	C179144-0001
18	07-Jul-10	C177787-0001	42	27-Jul-10	C178425-0001	66	17-Aug-10	C179145-0001
19	07-Jul-10	C177788-0001	43	28-Jul-10	C178499-0001	67	18-Aug-10	C179214-0001
20	07-Jul-10	C177789-0001	44	28-Jul-10	C178500-0001	68	18-Aug-10	C179215-0001
21	07-Jul-10	C177790-0001	45	29-Jul-10	C178525-0001	69	19-Aug-10	C179260-0001
22	07-Jul-10	C177791-0001	46	29-Jul-10	C178526-0001	70	19-Aug-10	C179261-0001
23	08-Jul-10	C177870-0001	47	03-Aug-10	C178585-0001	71	23-Aug-10	C179323-0001
24	12-Jul-10	C177946-0001	48	04-Aug-10	C178626-0001	72	24-Aug-10	C179337-0001

APPENDIX E

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
73	24-Aug-10	C179338-0001	97	13-Sep-10	C179835-0001	121	30-Sep-10	C180357-0001
74	24-Aug-10	C179339-0001	98	14-Sep-10	C179857-0001	122	04-Oct-10	C180409-0001
75	24-Aug-10	C179340-0001	99	14-Sep-10	C179858-0001	123	05-Oct-10	C180423-0001
76	25-Aug-10	C179386-0001	100	14-Sep-10	C179859-0001	124	05-Oct-10	C180424-0001
77	26-Aug-10	C179429-0001	101	14-Sep-10	C179860-0001	125	05-Oct-10	C180425-0001
78	26-Aug-10	C179430-0001	102	15-Sep-10	C179907-0001	126	05-Oct-10	C180426-0001
79	30-Aug-10	C179502-0001	103	16-Sep-10	C179956-0001	127	6-Oct-10	C180487-0001
80	31-Aug-10	C179524-0001	104	16-Sep-10	C179957-0001	128	6-Oct-10	C180488-0001
81	31-Aug-10	C179525-0001	105	20-Sep-10	C180015-0001	129	7-Oct-10	C180512-0001
82	31-Aug-10	C179526-0001	106	21-Sep-10	C180041-0001	130	12-Oct-10	C180608-0001
83	31-Aug-10	C179527-0001	107	21-Sep-10	C180042-0001	131	13-Oct-10	C180634-0001
84	01-Sep-10	C179566-0001	108	21-Sep-10	C180043-0001	132	13-Oct-10	C180635-0001
85	01-Sep-10	C179567-0001	109	21-Sep-10	C180044-0001	133	13-Oct-10	C180636-0001
86	01-Sep-10	C179591-0002	110	22-Sep-10	C180071-0001	134	13-Oct-10	C180637-0001
87	03-Sep-10	C179631-0001	111	23-Sep-10	C180113-0001	135	14-Oct-10	C180690-0001
88	03-Sep-10	C179636-0001	112	23-Sep-10	C180114-0001	136	14-Oct-10	C180691-0001
89	07-Sep-10	C179680-0001	113	23-Sep-10	C180126-0002	137	15-Oct-10	C180738-0001
90	08-Sep-10	C179716-0001	114	27-Sep-10	C180213-0001	138	19-Oct-10	C180833-0001
91	08-Sep-10	C179717-0001	115	28-Sep-10	C180260-0001	139	19-Oct-10	C180834-0001
92	08-Sep-10	C179718-0001	116	28-Sep-10	C180261-0001	140	19-Oct-10	C180835-0001
93	08-Sep-10	C179719-0001	117	28-Sep-10	C180262-0001	141	19-Oct-10	C180836-0001
94	09-Sep-10	C179740-0001	118	28-Sep-10	C180263-0001	142	19-Oct-10	C180837-0001
95	10-Sep-10	C179794-0001	119	29-Sep-10	C180275-0001	143	20-Oct-10	C180895-0001
96	13-Sep-10	C179834-0001	120	29-Sep-10	C180276-0001	144	20-Oct-10	C180896-0001

APPENDIX E

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
145	20-Oct-10	C180940-0001	147	25-Oct-10	C181048-0001			
146	21-Oct-10	C180958-0001	148	26-Oct-10	C181079-0001			
2010 WD samples tested by both ELISA and LC-MS/MS = 148								

TAELE E7. 2011 SURFACE WATER (WS) SAMPLES TESTED BY ELISA ONLY FOR MICROCYSTINS

Counter	Date	Sample I.D	Counter	Date	Sample I.D	Counter	Date	Sample I.D
1	28-Jul-11	C187164-0001	4	18-Aug-11	C187737-0002	7	29-Sep-11	C188898-0001
2	03-Aug-11	C187224-0001	5	09-Sep-11	C188325-0001			
3	18-Aug-11	C187737-0001	6	13-Sep-11	C188412-0001			
Total 2011 WS samples tested by ELISA only = 7								

TABLE E8. 2011 SURFACE WATER (WS) SAMPLES TESTED BY LC-MS/MS ONLY FOR MICROCYSTINS

Total 2011 WS samples tested by LC-MS/MS only = 0

TABLE E9. 2011 SURFACE WATER (WS) SAMPLES TESTED BY BOTH ELISA AND LC-MS/MS

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
1	07-Jun-11	C185590-0001	25	12-Jul-11	C186620-0001	49	15-Aug-11	C187600-0001
2	09-Jun-11	C185666-0001	26	12-Jul-11	C186620-0002	50	15-Aug-11	C187609-0001
3	09-Jun-11	C185666-0002	27	12-Jul-11	C186621-0001	51	15-Aug-11	C187609-0002
4	10-Jun-11	C185700-0001	28	19-Jul-11	C186812-0001	52	16-Aug-11	C187628-0001
5	10-Jun-11	C185700-0002	29	20-Jul-11	C186859-0001	53	17-Aug-11	C187658-0001
6	17-Jun-11	C185928-0001	30	26-Jul-11	C187010-0001	54	17-Aug-11	C187700-0001
7	21-Jun-11	C185986-0001	31	28-Jul-11	C187124-0001	55	17-Aug-11	C187701-0001
8	21-Jun-11	C185987-0001	32	28-Jul-11	C187124-0002	56	19-Aug-11	C187789-0001
9	21-Jun-11	C185988-0001	33	02-Aug-11	C187200-0001	57	19-Aug-11	C187790-0001
10	23-Jun-11	C186120-0001	34	02-Aug-11	C187200-0002	58	22-Aug-11	C187829-0001
11	23-Jun-11	C186120-0002	35	05-Aug-11	C187349-0001	59	22-Aug-11	C187829-0002
12	23-Jun-11	C186120-0003	36	05-Aug-11	C187349-0002	60	23-Aug-11	C187847-0001
13	23-Jun-11	C186120-0004	37	09-Aug-11	C187390-0001	61	26-Aug-11	C188001-0001
14	24-Jun-11	C186172-0001	38	09-Aug-11	C187390-0002	62	29-Aug-11	C188049-0001
15	28-Jun-11	C186241-0001	39	09-Aug-11	C187390-0003	63	30-Aug-11	C188063-0001
16	28-Jun-11	C186289-0001	40	09-Aug-11	C187404-0001	64	30-Aug-11	C188064-0001
17	28-Jun-11	C186289-0002	41	09-Aug-11	C187404-0002	65	31-Aug-11	C188145-0001
18	30-Jun-11	C186356-0001	42	09-Aug-11	C187405-0001	66	31-Aug-11	C188145-0002
19	30-Jun-11	C186356-0002	43	09-Aug-11	C187406-0001	67	07-Sep-11	C188252-0001
20	30-Jun-11	C186356-0003	44	09-Aug-11	C187406-0002	68	07-Sep-11	C188252-0002
21	05-Jul-11	C186430-0001	45	10-Aug-11	C187435-0001	69	07-Sep-11	C188252-0003
22	07-Jul-11	C186513-0001	46	10-Aug-11	C187435-0002	70	07-Sep-11	C188252-0004
23	08-Jul-11	C186561-0001	47	10-Aug-11	C187436-0001	71	07-Sep-11	C188252-0005
24	08-Jul-11	C186561-0002	48	12-Aug-11	C187565-0001	72	07-Sep-11	C188252-0006

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
73	08-Sep-11	C188297-0001	84	22-Sep-11	C188696-0001	95	12-Oct-11	C189233-0001
74	08-Sep-11	C188308-0001	85	27-Sep-11	C188786-0001	96	12-Oct-11	C189234-0001
75	08-Sep-11	C188308-0002	86	29-Sep-11	C188889-0001	97	13-Oct-11	C189268-0001
76	09-Sep-11	C188326-0001	87	29-Sep-11	C188890-0001	98	09-Nov-11	C190197-0001
77	09-Sep-11	C188326-0002	88	30-Sep-11	C188937-0001	99	09-Nov-11	C190198-0001
78	09-Sep-11	C188329-0001	89	30-Sep-11	C188938-0001	100	15-Nov-11	C190314-0001
79	13-Sep-11	C188394-0001	90	30-Sep-11	C188947-0001	101	15-Nov-11	C190314-0002
80	13-Sep-11	C188395-0001	91	06-Oct-11	C189063-0001	102	15-Nov-11	C190314-0003
81	21-Sep-11	C188624-0001	92	06-Oct-11	C189074-0001	103	15-Nov-11	C190314-0004
82	21-Sep-11	C188624-0002	93	06-Oct-11	C189075-0001	104	22-Nov-11	C190523-0001
83	21-Sep-11	C188624-0003	94	12-Oct-11	C189213-0001			
2011 WS samples tested by both LC-MS/MS & ELISA = 104								

TABLE E10. 2011 DRINKING WATER (WD) SAMPLES TESTED BY ELISA ONLY FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	14-Jun-11	C185799-0001	28	29-Jun-11	C186301-0001	55	13-Jul-11	C186658-0001
2	14-Jun-11	C185799-0002	29	29-Jun-11	C186301-0002	56	13-Jul-11	C186658-0002
3	15-Jun-11	C185820-0002	30	29-Jun-11	C186302-0001	57	13-Jul-11	C186659-0002
4	15-Jun-11	C185821-0002	31	29-Jun-11	C186302-0002	58	14-Jul-11	C186713-0002
5	15-Jun-11	C185822-0001	32	29-Jun-11	C186303-0002	59	14-Jul-11	C186714-0002
6	15-Jun-11	C185822-0002	33	29-Jun-11	C186304-0002	60	19-Jul-11	C186813-0001
7	16-Jun-11	C185896-0002	34	29-Jun-11	C186305-0002	61	19-Jul-11	C186813-0002
8	16-Jun-11	C185897-0001	35	30-Jun-11	C186384-0002	62	19-Jul-11	C186814-0001
9	16-Jun-11	C185897-0002	36	05-Jul-11	C186434-0001	63	19-Jul-11	C186814-0002
10	17-Jun-11	C185934-0002	37	05-Jul-11	C186434-0002	64	20-Jul-11	C186878-0002
11	21-Jun-11	C185990-0001	38	06-Jul-11	C186475-0002	65	20-Jul-11	C186879-0002
12	21-Jun-11	C185990-0002	39	06-Jul-11	C186476-0002	66	20-Jul-11	C186880-0002
13	21-Jun-11	C185991-0001	40	06-Jul-11	C186477-0002	67	20-Jul-11	C186881-0001
14	21-Jun-11	C185991-0002	41	06-Jul-11	C186478-0002	68	20-Jul-11	C186881-0002
15	22-Jun-11	C186067-0002	42	06-Jul-11	C186479-0002	69	21-Jul-11	C186916-0002
16	22-Jun-11	C186068-0001	43	06-Jul-11	C186487-0001	70	21-Jul-11	C186917-0002
17	22-Jun-11	C186068-0002	44	06-Jul-11	C186487-0002	71	25-Jul-11	C186990-0002
18	22-Jun-11	C186069-0002	45	07-Jul-11	C186531-0002	72	26-Jul-11	C187003-0002
19	23-Jun-11	C186125-0002	46	07-Jul-11	C186532-0001	73	26-Jul-11	C187004-0002
20	23-Jun-11	C186126-0002	47	07-Jul-11	C186532-0002	74	26-Jul-11	C187005-0001
21	27-Jun-11	C186226-0001	48	07-Jul-11	C186533-0002	75	26-Jul-11	C187005-0002
22	27-Jun-11	C186226-0002	49	12-Jul-11	C186605-0002	76	27-Jul-11	C187053-0002
23	27-Jun-11	C186226-0003	50	12-Jul-11	C186606-0002	77	27-Jul-11	C187054-0001
24	27-Jun-11	C186226-0004	51	12-Jul-11	C186607-0002	78	27-Jul-11	C187054-0002
25	28-Jun-11	C186262-0002	52	13-Jul-11	C186656-0002	79	27-Jul-11	C187055-0002
26	28-Jun-11	C186263-0001	53	13-Jul-11	C186657-0001	80	28-Jul-11	C187144-0002
27	28-Jun-11	C186263-0002	54	13-Jul-11	C186657-0002	81	28-Jul-11	C187145-0001

APPENDIX E

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
82	28-Jul-11	C187145-0002	110	12-Aug-11	C187559-0002	138	30-Aug-11	C188060-0001
83	28-Jul-11	C187146-0002	111	16-Aug-11	C187629-0001	139	30-Aug-11	C188060-0002
84	03-Aug-11	C187239-0001	112	16-Aug-11	C187629-0002	140	30-Aug-11	C188061-0002
85	03-Aug-11	C187239-0002	113	16-Aug-11	C187630-0002	141	31-Aug-11	C188127-0002
86	03-Aug-11	C187240-0002	114	17-Aug-11	C187664-0001	142	31-Aug-11	C188128-0001
87	03-Aug-11	C187241-0002	115	17-Aug-11	C187664-0002	143	31-Aug-11	C188128-0002
88	04-Aug-11	C187281-0002	116	17-Aug-11	C187665-0002	144	31-Aug-11	C188129-0002
89	04-Aug-11	C187282-0002	117	17-Aug-11	C187666-0002	145	31-Aug-11	C188130-0002
90	04-Aug-11	C187283-0001	118	17-Aug-11	C187667-0002	146	31-Aug-11	C188131-0002
91	04-Aug-11	C187283-0002	119	17-Aug-11	C187668-0001	147	31-Aug-11	C188132-0001
92	04-Aug-11	C187284-0002	120	17-Aug-11	C187668-0002	148	31-Aug-11	C188132-0002
93	04-Aug-11	C187285-0002	121	17-Aug-11	C187669-0002	149	01-Sep-11	C188170-0002
94	05-Aug-11	C187336-0001	122	18-Aug-11	C187740-0002	150	01-Sep-11	C188171-0002
95	05-Aug-11	C187336-0002	123	18-Aug-11	C187741-0002	151	02-Sep-11	C188210-0004
96	09-Aug-11	C187408-0002	124	18-Aug-11	C187742-0002	152	07-Sep-11	C188244-0002
97	09-Aug-11	C187409-0002	125	24-Aug-11	C187884-0002	153	07-Sep-11	C188245-0001
98	09-Aug-11	C187410-0001	126	24-Aug-11	C187885-0001	154	07-Sep-11	C188245-0002
99	09-Aug-11	C187410-0002	127	24-Aug-11	C187885-0002	155	08-Sep-11	C188291-0004
100	10-Aug-11	C187465-0001	128	24-Aug-11	C187886-0001	156	08-Sep-11	C188292-0003
101	10-Aug-11	C187465-0002	129	24-Aug-11	C187886-0002	157	08-Sep-11	C188292-0004
102	10-Aug-11	C187466-0002	130	24-Aug-11	C187887-0002	158	08-Sep-11	C188293-0004
103	10-Aug-11	C187467-0002	131	24-Aug-11	C187888-0001	159	08-Sep-11	C188294-0004
104	10-Aug-11	C187468-0002	132	24-Aug-11	C187888-0002	160	09-Sep-11	C188343-0002
105	11-Aug-11	C187494-0001	133	24-Aug-11	C187889-0002	161	09-Sep-11	C188344-0002
106	11-Aug-11	C187494-0002	134	24-Aug-11	C187890-0002	162	12-Sep-11	C188373-0002
107	11-Aug-11	C187495-0002	135	25-Aug-11	C187947-0002	163	14-Sep-11	C188435-0002
108	11-Aug-11	C187496-0002	136	26-Aug-11	C187946-0004	164	14-Sep-11	C188436-0002
109	12-Aug-11	C187558-0002	137	26-Aug-11	C188000-0002	165	14-Sep-11	C188437-0002

APPENDIX E

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
166	14-Sep-11	C188438-0002	194	28-Sep-11	C188823-0001	222	13-Oct-11	C189257-0002
167	14-Sep-11	C188439-0002	195	28-Sep-11	C188823-0002	223	13-Oct-11	C189258-0002
168	14-Sep-11	C188440-0001	196	28-Sep-11	C188824-0002	224	14-Oct-11	C189303-0002
169	14-Sep-11	C188440-0002	197	28-Sep-11	C188825-0002	225	14-Oct-11	C189304-0002
170	14-Sep-11	C188441-0001	198	29-Sep-11	C188906-0002	226	14-Oct-11	C189305-0002
171	14-Sep-11	C188441-0002	199	29-Sep-11	C188907-0002	227	14-Oct-11	C189306-0002
172	15-Sep-11	C188467-0002	200	29-Sep-11	C188908-0002	228	14-Oct-11	C189307-0001
173	16-Sep-11	C188511-0002	201	04-Oct-11	C188991-0002	229	14-Oct-11	C189307-0002
174	19-Sep-11	C188544-0002	202	04-Oct-11	C188992-0001	230	18-Oct-11	C189392-0001
175	20-Sep-11	C188586-0002	203	04-Oct-11	C188992-0002	231	18-Oct-11	C189392-0002
176	21-Sep-11	C188631-0002	204	04-Oct-11	C188993-0001	232	18-Oct-11	C189393-0001
177	21-Sep-11	C188632-0002	205	04-Oct-11	C188993-0002	233	18-Oct-11	C189393-0002
178	21-Sep-11	C188633-0002	206	05-Oct-11	C189035-0001	234	19-Oct-11	C189473-0001
179	21-Sep-11	C188634-0002	207	05-Oct-11	C189035-0002	235	19-Oct-11	C189473-0002
180	21-Sep-11	C188635-0001	208	05-Oct-11	C189036-0002	236	19-Oct-11	C189474-0001
181	21-Sep-11	C188635-0002	209	05-Oct-11	C189037-0001	237	19-Oct-11	C189474-0002
182	21-Sep-11	C188636-0001	210	05-Oct-11	C189038-0002	238	19-Oct-11	C189475-0001
183	21-Sep-11	C188636-0002	211	05-Oct-11	C189039-0002	239	19-Oct-11	C189475-0002
184	22-Sep-11	C188683-0002	212	06-Oct-11	C189068-0002	240	19-Oct-11	C189476-0002
185	22-Sep-11	C188684-0002	213	06-Oct-11	C189069-0002	241	19-Oct-11	C189477-0001
186	23-Sep-11	C188723-0002	214	06-Oct-11	C189070-0002	242	19-Oct-11	C189477-0002
187	26-Sep-11	C188766-0002	215	07-Oct-11	C189136-0002	243	19-Oct-11	C189481-0001
188	28-Sep-11	C188819-0002	216	12-Oct-11	C189205-0002	244	19-Oct-11	C189481-0002
189	28-Sep-11	C188819-0003	217	12-Oct-11	C189206-0001	245	20-Oct-11	C189574-0001
190	28-Sep-11	C188820-0002	218	12-Oct-11	C189206-0002	246	20-Oct-11	C189574-0002
191	28-Sep-11	C188821-0001	219	13-Oct-11	C189255-0002	247	20-Oct-11	C189575-0001
192	28-Sep-11	C188821-0002	220	13-Oct-11	C189256-0002	248	20-Oct-11	C189575-0002
193	28-Sep-11	C188822-0002	221	13-Oct-11	C189257-0001	249	21-Oct-11	C189612-0001

APPENDIX E

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
250	21-Oct-11	C189612-0002	278	04-Nov-11	C190072-0002	306	17-Nov-11	C190448-0002
251	25-Oct-11	C189681-0001	279	08-Nov-11	C190145-0001	307	22-Nov-11	C190505-0001
252	25-Oct-11	C189681-0002	280	08-Nov-11	C190145-0002	308	22-Nov-11	C190505-0002
253	26-Oct-11	C189738-0001	281	08-Nov-11	C190146-0001	309	23-Nov-11	C190578-0001
254	26-Oct-11	C189738-0002	282	08-Nov-11	C190146-0002	310	23-Nov-11	C190578-0002
255	26-Oct-11	C189739-0001	283	09-Nov-11	C190207-0002	311	23-Nov-11	C190579-0002
256	26-Oct-11	C189739-0002	284	09-Nov-11	C190208-0001	312	23-Nov-11	C190580-0002
257	26-Oct-11	C189740-0002	285	09-Nov-11	C190208-0002	313	23-Nov-11	C190581-0001
258	26-Oct-11	C189741-0001	286	09-Nov-11	C190209-0002	314	23-Nov-11	C190581-0002
259	26-Oct-11	C189741-0002	287	09-Nov-11	C190210-0002	315	23-Nov-11	C190582-0002
260	26-Oct-11	C189742-0002	288	10-Nov-11	C190234-0002	316	23-Nov-11	C190583-0002
261	26-Oct-11	C189743-0002	289	10-Nov-11	C190236-0002	317	23-Nov-11	C190584-0002
262	26-Oct-11	C189744-0002	290	10-Nov-11	C190237-0002	318	24-Nov-11	C190607-0004
263	27-Oct-11	C189794-0002	291	14-Nov-11	C190291-0002	319	24-Nov-11	C190609-0002
264	28-Oct-11	C189855-0002	292	14-Nov-11	C190292-0002	320	29-Nov-11	C190719-0001
265	31-Oct-11	C189894-0002	293	15-Nov-11	C190321-0001	321	29-Nov-11	C190719-0002
266	01-Nov-11	C189922-0001	294	15-Nov-11	C190321-0002	322	30-Nov-11	C190761-0002
267	01-Nov-11	C189922-0002	295	16-Nov-11	C190408-0002	323	30-Nov-11	C190762-0002
268	01-Nov-11	C189923-0001	296	16-Nov-11	C190409-0002	324	30-Nov-11	C190763-0002
269	01-Nov-11	C189923-0002	297	16-Nov-11	C190410-0001	325	30-Nov-11	C190764-0001
270	02-Nov-11	C189970-0002	298	16-Nov-11	C190410-0002	326	30-Nov-11	C190764-0002
271	02-Nov-11	C189971-0002	299	16-Nov-11	C190411-0001	327	30-Nov-11	C190765-0001
272	02-Nov-11	C189972-0001	300	16-Nov-11	C190411-0002	328	30-Nov-11	C190765-0002
273	02-Nov-11	C189972-0002	301	16-Nov-11	C190412-0002	329	30-Nov-11	C190766-0001
274	03-Nov-11	C190025-0002	302	16-Nov-11	C190413-0002	330	30-Nov-11	C190766-0002
275	03-Nov-11	C190026-0002	303	17-Nov-11	C190445-0002	331	01-Dec-11	C190798-0002
276	03-Nov-11	C190027-0002	304	17-Nov-11	C190446-0002	332	01-Dec-11	C190799-0002
277	04-Nov-11	C190071-0002	305	17-Nov-11	C190447-0002	333	01-Dec-11	C190800-0002

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
334	05-Dec-11	C190881-0002	335	15-Dec-11	C191139-0002			
2011 WD samples tested by ELISA only = 335								

TABLE E11. 2011 DRINKING WATER (WD) SAMPLES TESTED LC-MS/MS ONLY FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	14-Jun-11	C185806-0001	5	14-Jun-11	C185806-0005	9	27-Jul-11	C187052-0004
2	14-Jun-11	C185806-0002	6	27-Jul-11	C187052-0001	10	27-Jul-11	C187052-0005
3	14-Jun-11	C185806-0003	7	27-Jul-11	C187052-0002			
4	14-Jun-11	C185806-0004	8	27-Jul-11	C187052-0003			
2011 WD samples tested by LC-MS/MS only = 10								

TABLE E12. 2011 DRINKING WATER (WD) SAMPLES TESTED BY BOTH ELISA AND LC-MS/MS

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
1	15-Jun-11	C185820-0001	25	13-Jul-11	C186659-0001	49	10-Aug-11	C187468-0001
2	15-Jun-11	C185821-0001	26	14-Jul-11	C186713-0001	50	11-Aug-11	C187495-0001
3	16-Jun-11	C185896-0001	27	20-Jul-11	C186878-0001	51	11-Aug-11	C187496-0001
4	17-Jun-11	C185934-0001	28	20-Jul-11	C186879-0001	52	12-Aug-11	C187558-0001
5	22-Jun-11	C186067-0001	29	20-Jul-11	C186880-0001	53	12-Aug-11	C187559-0001
6	22-Jun-11	C186069-0001	30	21-Jul-11	C186916-0001	54	16-Aug-11	C187630-0001
7	23-Jun-11	C186125-0001	31	21-Jul-11	C186917-0001	55	17-Aug-11	C187665-0001
8	23-Jun-11	C186126-0001	32	26-Jul-11	C187003-0001	56	17-Aug-11	C187666-0001
9	28-Jun-11	C186262-0001	33	26-Jul-11	C187004-0001	57	17-Aug-11	C187667-0001
10	29-Jun-11	C186303-0001	34	27-Jul-11	C187053-0001	58	17-Aug-11	C187669-0001
11	29-Jun-11	C186304-0001	35	27-Jul-11	C187055-0001	59	18-Aug-11	C187740-0001
12	29-Jun-11	C186305-0001	36	28-Jul-11	C187144-0001	60	18-Aug-11	C187741-0001
13	30-Jun-11	C186384-0001	37	28-Jul-11	C187146-0001	61	18-Aug-11	C187742-0001
14	06-Jul-11	C186475-0001	38	03-Aug-11	C187240-0001	62	23-Aug-11	C187841-0001
15	06-Jul-11	C186476-0001	39	03-Aug-11	C187241-0001	63	23-Aug-11	C187841-0002
16	06-Jul-11	C186477-0001	40	04-Aug-11	C187281-0001	64	24-Aug-11	C187884-0001
17	06-Jul-11	C186478-0001	41	04-Aug-11	C187282-0001	65	24-Aug-11	C187887-0001
18	06-Jul-11	C186479-0001	42	04-Aug-11	C187284-0001	66	24-Aug-11	C187889-0001
19	07-Jul-11	C186531-0001	43	04-Aug-11	C187285-0001	67	24-Aug-11	C187890-0001
20	07-Jul-11	C186533-0001	44	09-Aug-11	C187389-0001	68	25-Aug-11	C187947-0001
21	12-Jul-11	C186605-0001	45	09-Aug-11	C187408-0001	69	26-Aug-11	C187946-0003
22	12-Jul-11	C186606-0001	46	09-Aug-11	C187409-0001	70	26-Aug-11	C188000-0001
23	12-Jul-11	C186607-0001	47	10-Aug-11	C187466-0001	71	30-Aug-11	C188061-0001
24	13-Jul-11	C186656-0001	48	10-Aug-11	C187467-0001	72	31-Aug-11	C188127-0001

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by ELISA	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
73	31-Aug-11	C188129-0001	101	21-Sep-11	C188634-0001	129	26-Oct-11	C189740-0001
74	31-Aug-11	C188130-0001	102	22-Sep-11	C188683-0001	130	26-Oct-11	C189742-0001
75	31-Aug-11	C188131-0001	103	22-Sep-11	C188684-0001	131	26-Oct-11	C189743-0001
76	01-Sep-11	C188170-0001	104	23-Sep-11	C188723-0001	132	26-Oct-11	C189744-0001
77	01-Sep-11	C188171-0001	105	26-Sep-11	C188766-0001	133	27-Oct-11	C189794-0001
78	02-Sep-11	C188210-0003	106	28-Sep-11	C188820-0001	134	28-Oct-11	C189855-0001
79	07-Sep-11	C188244-0001	107	28-Sep-11	C188822-0001	135	31-Oct-11	C189894-0001
80	08-Sep-11	C188291-0003	108	28-Sep-11	C188824-0001	136	02-Nov-11	C189970-0001
81	08-Sep-11	C188293-0003	109	28-Sep-11	C188825-0001	137	02-Nov-11	C189971-0001
82	08-Sep-11	C188294-0003	110	29-Sep-11	C188906-0001	138	03-Nov-11	C190025-0001
83	08-Sep-11	C188295-0002	111	29-Sep-11	C188907-0001	139	03-Nov-11	C190026-0001
84	09-Sep-11	C188343-0001	112	29-Sep-11	C188908-0001	140	03-Nov-11	C190027-0001
85	09-Sep-11	C188344-0001	113	04-Oct-11	C188991-0001	141	04-Nov-11	C190071-0001
86	12-Sep-11	C188373-0001	114	05-Oct-11	C189036-0001	142	04-Nov-11	C190072-0001
87	13-Sep-11	C188388-0001	115	05-Oct-11	C189038-0001	143	09-Nov-11	C190207-0001
88	13-Sep-11	C188388-0002	116	05-Oct-11	C189039-0001	144	09-Nov-11	C190209-0001
89	14-Sep-11	C188435-0001	117	06-Oct-11	C189068-0001	145	09-Nov-11	C190210-0001
90	14-Sep-11	C188436-0001	118	06-Oct-11	C189069-0001	146	10-Nov-11	C190234-0001
91	14-Sep-11	C188437-0001	119	06-Oct-11	C189070-0001	147	10-Nov-11	C190236-0001
92	14-Sep-11	C188438-0001	120	07-Oct-11	C189136-0001	148	10-Nov-11	C190237-0001
93	14-Sep-11	C188439-0001	121	12-Oct-11	C189205-0001	149	14-Nov-11	C190291-0001
94	15-Sep-11	C188467-0001	122	13-Oct-11	C189255-0001	150	14-Nov-11	C190292-0001
95	16-Sep-11	C188511-0001	123	13-Oct-11	C189256-0001	151	16-Nov-11	C190408-0001
96	19-Sep-11	C188544-0001	124	13-Oct-11	C189258-0001	152	16-Nov-11	C190409-0001
97	20-Sep-11	C188586-0001	125	14-Oct-11	C189303-0001	153	16-Nov-11	C190412-0001
98	21-Sep-11	C188631-0001	126	14-Oct-11	C189304-0001	154	16-Nov-11	C190413-0001
99	21-Sep-11	C188632-0001	127	14-Oct-11	C189305-0001	155	17-Nov-11	C190445-0001
100	21-Sep-11	C188633-0001	128	14-Oct-11	C189306-0001	156	17-Nov-11	C190446-0001

APPENDIX E

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
157	17-Nov-11	C190448-0001	163	24-Nov-11	C190607-0003	169	01-Dec-11	C190799-0001
158	23-Nov-11	C190579-0001	164	24-Nov-11	C190609-0001	170	01-Dec-11	C190800-0001
159	23-Nov-11	C190580-0001	165	30-Nov-11	C190761-0001	171	05-Dec-11	C190881-0001
160	23-Nov-11	C190582-0001	166	30-Nov-11	C190762-0001	172	15-Dec-11	C191139-0001
161	23-Nov-11	C190583-0001	167	30-Nov-11	C190763-0001			
162	23-Nov-11	C190584-0001	168	01-Dec-11	C190798-0001			
2011 drinking water (WD) samples tested by both ELISA and LC-MS/MS = 172								

TABLE E13. 2012 SURFACE WATER (WS) SAMPLES TESTED BY ELISA ONLY FOR MICROCYSTINS

Counter	Date	Sample I.D	Counter	Date	Sample I.D	Counter	Date	Sample I.D
1	11-Jun-12	C194599-0001	21	28-Sep-12	C197602-0002	41	28-Sep-12	C197603-0015
2	20-Jun-12	C194923-0002	22	28-Sep-12	C197602-0003	42	28-Sep-12	C197603-0017
3	29-Jun-12	C195177-0005	23	28-Sep-12	C197602-0005	43	28-Sep-12	C197603-0019
4	29-Jun-12	C195177-0006	24	28-Sep-12	C197602-0006	44	28-Sep-12	C197603-0021
5	29-Jun-12	C195177-0007	25	28-Sep-12	C197602-0008	45	28-Sep-12	C197603-0023
6	03-Jul-12	C195194-0001	26	28-Sep-12	C197602-0009	46	28-Sep-12	C197603-0024
7	17-Jul-12	C195551-0001	27	28-Sep-12	C197602-0011	47	28-Sep-12	C197603-0026
8	24-Jul-12	C195774-0001	28	28-Sep-12	C197602-0013	48	28-Sep-12	C197603-0027
9	27-Jul-12	C195915-0001	29	28-Sep-12	C197602-0015	49	05-Oct-12	C197771-0001
10	27-Jul-12	C195915-0002	30	28-Sep-12	C197602-0017	50	05-Oct-12	C197771-0003
11	07-Aug-12	C196149-0001	31	28-Sep-12	C197602-0018	51	05-Oct-12	C197771-0005
12	07-Aug-12	C196149-0002	32	28-Sep-12	C197602-0021	52	05-Oct-12	C197771-0019
13	14-Aug-12	C196353-0002	33	28-Sep-12	C197602-0023	53	05-Oct-12	C197771-0021
14	05-Sep-12	C196885-0001	34	28-Sep-12	C197603-0002	54	05-Oct-12	C197771-0028
15	05-Sep-12	C196885-0002	35	28-Sep-12	C197603-0004	55	05-Oct-12	C197772-0007
16	07-Sep-12	C197008-0005	36	28-Sep-12	C197603-0006	56	05-Oct-12	C197772-0009
17	07-Sep-12	C197008-0010	37	28-Sep-12	C197603-0008	57	05-Oct-12	C197772-0011
18	07-Sep-12	C197010-0002	38	28-Sep-12	C197603-0010	58	05-Oct-12	C197772-0021
19	19-Sep-12	C197312-0001	39	28-Sep-12	C197603-0012	59	14-Nov-12	C198934-0001
20	28-Sep-12	C197602-0001	40	28-Sep-12	C197603-0013			
Total 2012 WS samples tested by ELISA only = 59								

TABLE E14. 2012 SURFACE WATER (WS) SAMPLES TESTED BY LC-MS/MS ONLY FOR MICROCYSTINS

Counter	Date	Sample I.D	Counter	Date	Sample I.D	Counter	Date	Sample I.D
1	17-Aug-12	C196511-0001	5	31-Aug-12	C196877-0015	9	05-Oct-12	C197771-0015
2	31-Aug-12	C196876-0007	6	31-Aug-12	C196877-0019	10	05-Oct-12	C197772-0001
3	31-Aug-12	C196876-0017	7	07-Sep-12	C197010-0011	11	05-Oct-12	C197772-0015
4	31-Aug-12	C196877-0005	8	07-Sep-12	C197010-0023			
2012 WS samples tested by LC-MS/MS only = 11								

TABLE E15. 2012 SURFACE WATER (WS) SAMPLES TESTED BY BOTH ELISA AND LC-MS/MS

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
1	13-Apr-12	C193172-0001	25	30-Jul-12	C195937-0001	49	10-Aug-12	C196275-0001
2	26-Apr-12	C193446-0001	26	31-Jul-12	C195974-0001	50	14-Aug-12	C196332-0001
3	11-May-12	C193850-0006	27	31-Jul-12	C195979-0001	51	14-Aug-12	C196336-0001
4	07-Jun-12	C194521-0001	28	31-Jul-12	C195979-0002	52	14-Aug-12	C196337-0001
5	07-Jun-12	C194522-0001	29	31-Jul-12	C195979-0003	53	14-Aug-12	C196337-0002
6	13-Jun-12	C194683-0001	30	01-Aug-12	C196026-0001	54	14-Aug-12	C196353-0001
7	20-Jun-12	C194907-0001	31	01-Aug-12	C196026-0002	55	15-Aug-12	C196413-0001
8	20-Jun-12	C194923-0001	32	01-Aug-12	C196027-0001	56	15-Aug-12	C196413-0002
9	26-Jun-12	C195037-0001	33	01-Aug-12	C196029-0001	57	15-Aug-12	C196413-0003
10	28-Jun-12	C195159-0001	34	01-Aug-12	C196032-0001	58	15-Aug-12	C196413-0004
11	28-Jun-12	C195159-0002	35	01-Aug-12	C196032-0002	59	15-Aug-12	C196414-0001
12	29-Jun-12	C195179-0001	36	03-Aug-12	C196114-0001	60	16-Aug-12	C196458-0001
13	04-Jul-12	C195240-0001	37	03-Aug-12	C196124-0001	61	16-Aug-12	C196459-0001
14	04-Jul-12	C195240-0002	38	03-Aug-12	C196124-0002	62	17-Aug-12	C196493-0001
15	11-Jul-12	C195448-0001	39	03-Aug-12	C196130-0001	63	17-Aug-12	C196493-0002
16	12-Jul-12	C195486-0001	40	07-Aug-12	C196149-0003	64	20-Aug-12	C196527-0001
17	12-Jul-12	C195486-0002	41	07-Aug-12	C196149-0004	65	20-Aug-12	C196527-0002
18	12-Jul-12	C195486-0003	42	08-Aug-12	C196195-0001	66	20-Aug-12	C196532-0001
19	17-Jul-12	C195580-0001	43	08-Aug-12	C196196-0001	67	20-Aug-12	C196532-0002
20	18-Jul-12	C195637-0001	44	08-Aug-12	C196196-0002	68	21-Aug-12	C196548-0001
21	18-Jul-12	C195637-0002	45	08-Aug-12	C196200-0001	69	21-Aug-12	C196549-0001
22	19-Jul-12	C195677-0001	46	08-Aug-12	C196200-0002	70	22-Aug-12	C196586-0001
23	20-Jul-12	C195726-0001	47	08-Aug-12	C196200-0003	71	22-Aug-12	C196586-0002
24	26-Jul-12	C195859-0001	48	10-Aug-12	C196274-0001	72	22-Aug-12	C196586-0003

APPENDIX E

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
73	22-Aug-12	C196586-0004	94	05-Sep-12	C196897-0001	115	14-Sep-12	C197228-0003
74	22-Aug-12	C196587-0001	95	05-Sep-12	C196897-0002	116	14-Sep-12	C197228-0004
75	22-Aug-12	C196587-0002	96	06-Sep-12	C196944-0001	117	14-Sep-12	C197229-0001
76	22-Aug-12	C196587-0003	97	06-Sep-12	C196956-0001	118	14-Sep-12	C197229-0002
77	22-Aug-12	C196588-0001	98	07-Sep-12	C196989-0001	119	18-Sep-12	C197271-0001
78	22-Aug-12	C196592-0001	99	07-Sep-12	C196997-0001	120	20-Sep-12	C197374-0001
79	22-Aug-12	C196592-0001	100	07-Sep-12	C196997-0002	121	20-Sep-12	C197375-0001
80	22-Aug-12	C196592-0002	101	07-Sep-12	C196998-0001	122	20-Sep-12	C197376-0001
81	22-Aug-12	C196605-0001	102	07-Sep-12	C196999-0001	123	21-Sep-12	C197394-0001
82	22-Aug-12	C196605-0002	103	07-Sep-12	C196999-0002	124	21-Sep-12	C197394-0002
83	22-Aug-12	C196605-0003	104	07-Sep-12	C196999-0003	125	27-Sep-12	C197578-0001
84	23-Aug-12	C196641-0001	105	07-Sep-12	C197008-0012	126	27-Sep-12	C197578-0002
85	23-Aug-12	C196652-0004	106	11-Sep-12	C197056-0001	127	03-Oct-12	C197700-0001
86	28-Aug-12	C196742-0001	107	13-Sep-12	C197190-0001	128	05-Oct-12	C197772-0012
87	28-Aug-12	C196752-0001	108	13-Sep-12	C197190-0002	129	10-Oct-12	C197826-0001
88	28-Aug-12	C196752-0002	109	13-Sep-12	C197190-0003	130	11-Oct-12	C197894-0001
89	28-Aug-12	C196752-0003	110	14-Sep-12	C197218-0001	131	19-Oct-12	C198208-0001
90	28-Aug-12	C196752-0004	111	14-Sep-12	C197218-0002	132	25-Oct-12	C198407-0001
91	29-Aug-12	C196759-0001	112	14-Sep-12	C197220-0001	133	31-Oct-12	C198562-0001
92	29-Aug-12	C196759-0002	113	14-Sep-12	C197228-0001	134	14-Nov-12	C198934-0001
93	31-Aug-12	C196872-0001	114	14-Sep-12	C197228-0002			
2012 WS samples tested by both ELISA and LC-MS/MS = 134								

TABLE E16. 2012 DRINKING WATER (WD) SAMPLES TESTED BY ELISA ONLY FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	12-Jan-12	C191512-0002	28	13-Jun-12	C194678-0002	55	21-Jun-12	C194944-0002
2	19-Jan-12	C191643-0002	29	13-Jun-12	C194679-0001	56	22-Jun-12	C194983-0001
3	02-Feb-12	C191888-0002	30	13-Jun-12	C194679-0002	57	22-Jun-12	C194983-0002
4	16-Feb-12	C192116-0002	31	14-Jun-12	C194719-0002	58	26-Jun-12	C195013-0001
5	01-Mar-12	C192364-0002	32	14-Jun-12	C194720-0001	59	26-Jun-12	C195013-0002
6	15-Mar-12	C192647-0002	33	14-Jun-12	C194720-0002	60	27-Jun-12	C195081-0002
7	29-Mar-12	C192912-0002	34	14-Jun-12	C194728-0001	61	27-Jun-12	C195082-0001
8	12-Apr-12	C193110-0002	35	14-Jun-12	C194728-0002	62	27-Jun-12	C195082-0002
9	26-Apr-12	C193451-0002	36	19-Jun-12	C194811-0001	63	27-Jun-12	C195083-0001
10	02-May-12	C193549-0001	37	19-Jun-12	C194811-0002	64	27-Jun-12	C195083-0002
11	02-May-12	C193549-0002	38	19-Jun-12	C194812-0001	65	27-Jun-12	C195084-0001
12	02-May-12	C193549-0003	39	19-Jun-12	C194812-0002	66	27-Jun-12	C195084-0002
13	02-May-12	C193549-0004	40	20-Jun-12	C194884-0002	67	27-Jun-12	C195085-0002
14	10-May-12	C193785-0002	41	20-Jun-12	C194885-0001	68	27-Jun-12	C195086-0001
15	24-May-12	C194131-0002	42	20-Jun-12	C194885-0002	69	27-Jun-12	C195086-0002
16	07-Jun-12	C194508-0002	43	20-Jun-12	C194886-0002	70	27-Jun-12	C195087-0002
17	12-Jun-12	C194640-0001	44	20-Jun-12	C194887-0002	71	27-Jun-12	C195088-0001
18	12-Jun-12	C194640-0002	45	20-Jun-12	C194888-0001	72	27-Jun-12	C195088-0002
19	13-Jun-12	C194672-0001	46	20-Jun-12	C194888-0002	73	28-Jun-12	C195123-0001
20	13-Jun-12	C194672-0002	47	20-Jun-12	C194889-0002	74	28-Jun-12	C195123-0002
21	13-Jun-12	C194673-0001	48	20-Jun-12	C194890-0001	75	28-Jun-12	C195126-0001
22	13-Jun-12	C194673-0002	49	20-Jun-12	C194890-0002	76	28-Jun-12	C195126-0002
23	13-Jun-12	C194674-0002	50	20-Jun-12	C194919-0001	77	28-Jun-12	C195127-0002
24	13-Jun-12	C194675-0001	51	20-Jun-12	C194919-0002	78	04-Jul-12	C195209-0001
25	13-Jun-12	C194675-0002	52	21-Jun-12	C194943-0001	79	04-Jul-12	C195209-0002
26	13-Jun-12	C194677-0002	53	21-Jun-12	C194943-0002	80	04-Jul-12	C195210-0002
27	13-Jun-12	C194678-0001	54	21-Jun-12	C194944-0001	81	04-Jul-12	C195211-0001

APPENDIX E

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
82	04-Jul-12	C195211-0002	110	11-Jul-12	C195429-0002	138	23-Jul-12	C195755-0004
83	04-Jul-12	C195214-0001	111	11-Jul-12	C195430-0001	139	23-Jul-12	C195755-0005
84	04-Jul-12	C195214-0002	112	12-Jul-12	C195468-0002	140	24-Jul-12	C195765-0001
85	05-Jul-12	C195268-0001	113	13-Jul-12	C195512-0001	141	24-Jul-12	C195765-0002
86	05-Jul-12	C195268-0002	114	13-Jul-12	C195512-0002	142	24-Jul-12	C195766-0001
87	05-Jul-12	C195269-0002	115	13-Jul-12	C195513-0001	143	24-Jul-12	C195766-0002
88	05-Jul-12	C195270-0002	116	13-Jul-12	C195513-0002	144	25-Jul-12	C195835-0001
89	05-Jul-12	C195271-0002	117	17-Jul-12	C195552-0001	145	25-Jul-12	C195835-0002
90	05-Jul-12	C195272-0001	118	17-Jul-12	C195552-0002	146	25-Jul-12	C195836-0002
91	05-Jul-12	C195272-0002	119	17-Jul-12	C195553-0001	147	25-Jul-12	C195837-0001
92	05-Jul-12	C195273-0001	120	17-Jul-12	C195553-0002	148	25-Jul-12	C195837-0002
93	05-Jul-12	C195273-0002	121	18-Jul-12	C195613-0002	149	25-Jul-12	C195838-0001
94	05-Jul-12	C195274-0001	122	18-Jul-12	C195614-0001	150	25-Jul-12	C195838-0002
95	05-Jul-12	C195274-0002	123	18-Jul-12	C195614-0002	151	25-Jul-12	C195839-0002
96	06-Jul-12	C195303-0001	124	18-Jul-12	C195615-0002	152	25-Jul-12	C195840-0001
97	06-Jul-12	C195303-0002	125	18-Jul-12	C195616-0001	153	25-Jul-12	C195840-0002
98	10-Jul-12	C195366-0001	126	18-Jul-12	C195616-0002	154	25-Jul-12	C195841-0002
99	10-Jul-12	C195366-0002	127	18-Jul-12	C195617-0001	155	26-Jul-12	C195868-0001
100	10-Jul-12	C195367-0001	128	18-Jul-12	C195617-0002	156	26-Jul-12	C195868-0002
101	10-Jul-12	C195367-0002	129	18-Jul-12	C195618-0002	157	26-Jul-12	C195869-0001
102	11-Jul-12	C195424-0001	130	18-Jul-12	C195619-0002	158	26-Jul-12	C195869-0002
103	11-Jul-12	C195424-0002	131	18-Jul-12	C195620-0001	159	31-Jul-12	C195957-0001
104	11-Jul-12	C195425-0002	132	18-Jul-12	C195620-0002	160	31-Jul-12	C195957-0002
105	11-Jul-12	C195426-0001	133	18-Jul-12	C195621-0001	161	01-Aug-12	C196003-0002
106	11-Jul-12	C195426-0002	134	18-Jul-12	C195621-0002	162	01-Aug-12	C196004-0001
107	11-Jul-12	C195427-0001	135	23-Jul-12	C195755-0001	163	01-Aug-12	C196004-0002
108	11-Jul-12	C195427-0002	136	23-Jul-12	C195755-0002	164	01-Aug-12	C196005-0002
109	11-Jul-12	C195428-0002	137	23-Jul-12	C195755-0003	165	01-Aug-12	C196006-0001

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
166	01-Aug-12	C196006-0002	194	15-Aug-12	C196402-0002	222	29-Aug-12	C196769-0002
167	01-Aug-12	C196007-0001	195	15-Aug-12	C196403-0002	223	29-Aug-12	C196770-0002
168	01-Aug-12	C196007-0002	196	15-Aug-12	C196404-0002	224	29-Aug-12	C196771-0002
169	01-Aug-12	C196008-0002	197	15-Aug-12	C196405-0002	225	29-Aug-12	C196772-0002
170	01-Aug-12	C196009-0002	198	15-Aug-12	C196406-0002	226	29-Aug-12	C196773-0002
171	01-Aug-12	C196010-0001	199	15-Aug-12	C196407-0002	227	29-Aug-12	C196774-0002
172	01-Aug-12	C196010-0002	200	15-Aug-12	C196408-0002	228	29-Aug-12	C196775-0002
173	02-Aug-12	C196097-0001	201	15-Aug-12	C196409-0002	229	31-Aug-12	C196869-0002
174	02-Aug-12	C196097-0002	202	17-Aug-12	C196495-0002	230	05-Sep-12	C196889-0002
175	02-Aug-12	C196098-0001	203	21-Aug-12	C196542-0002	231	05-Sep-12	C196890-0002
176	02-Aug-12	C196098-0002	204	21-Aug-12	C196543-0002	232	05-Sep-12	C196891-0002
177	02-Aug-12	C196099-0001	205	21-Aug-12	C196544-0002	233	06-Sep-12	C196945-0002
178	02-Aug-12	C196099-0002	206	22-Aug-12	C196608-0002	234	06-Sep-12	C196946-0002
179	08-Aug-12	C196173-0002	207	22-Aug-12	C196609-0002	235	06-Sep-12	C196947-0002
180	08-Aug-12	C196174-0002	208	22-Aug-12	C196610-0002	236	06-Sep-12	C196948-0002
181	09-Aug-12	C196239-0002	209	22-Aug-12	C196613-0002	237	06-Sep-12	C196949-0002
182	09-Aug-12	C196240-0002	210	22-Aug-12	C196614-0002	238	07-Sep-12	C196980-0002
183	09-Aug-12	C196241-0002	211	22-Aug-12	C196615-0002	239	07-Sep-12	C196981-0002
184	09-Aug-12	C196242-0002	212	22-Aug-12	C196616-0002	240	07-Sep-12	C196982-0002
185	09-Aug-12	C196243-0002	213	22-Aug-12	C196617-0002	241	10-Sep-12	C197019-0002
186	09-Aug-12	C196244-0002	214	23-Aug-12	C196637-0002	242	11-Sep-12	C197053-0002
187	09-Aug-12	C196245-0002	215	28-Aug-12	C196731-0002	243	12-Sep-12	C197139-0002
188	09-Aug-12	C196246-0002	216	28-Aug-12	C196748-0001	244	12-Sep-12	C197140-0002
189	09-Aug-12	C196247-0002	217	28-Aug-12	C196748-0002	245	12-Sep-12	C197141-0002
190	10-Aug-12	C196268-0002	218	28-Aug-12	C196748-0003	246	12-Sep-12	C197142-0002
191	14-Aug-12	C196334-0002	219	28-Aug-12	C196748-0004	247	12-Sep-12	C197143-0002
192	15-Aug-12	C196400-0002	220	29-Aug-12	C196730-0003	248	12-Sep-12	C197144-0002
193	15-Aug-12	C196401-0002	221	29-Aug-12	C196767-0002	249	12-Sep-12	C197145-0002

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
250	12-Sep-12	C197146-0002	278	02-Oct-12	C197645-0002	306	17-Oct-12	C198071-0002
251	12-Sep-12	C197147-0002	279	02-Oct-12	C197648-0002	307	17-Oct-12	C198072-0002
252	13-Sep-12	C197185-0002	280	02-Oct-12	C197649-0002	308	17-Oct-12	C198073-0002
253	18-Sep-12	C197264-0002	281	03-Oct-12	C197689-0002	309	17-Oct-12	C198074-0002
254	19-Sep-12	C197326-0002	282	03-Oct-12	C197690-0002	310	17-Oct-12	C198075-0002
255	19-Sep-12	C197327-0002	283	03-Oct-12	C197691-0002	311	17-Oct-12	C198076-0002
256	19-Sep-12	C197328-0002	284	03-Oct-12	C197692-0002	312	18-Oct-12	C198126-0002
257	19-Sep-12	C197329-0002	285	03-Oct-12	C197693-0002	313	18-Oct-12	C198127-0002
258	19-Sep-12	C197330-0002	286	03-Oct-12	C197694-0002	314	19-Oct-12	C198190-0002
259	19-Sep-12	C197331-0002	287	03-Oct-12	C197695-0002	315	23-Oct-12	C198267-0002
260	19-Sep-12	C197332-0002	288	04-Oct-12	C197741-0002	316	24-Oct-12	C198325-0002
261	19-Sep-12	C197333-0002	289	05-Oct-12	C197779-0002	317	24-Oct-12	C198326-0002
262	19-Sep-12	C197334-0002	290	09-Oct-12	C197802-0002	318	24-Oct-12	C198327-0001
263	20-Sep-12	C197368-0002	291	10-Oct-12	C197819-0002	319	24-Oct-12	C198327-0002
264	20-Sep-12	C197369-0002	292	10-Oct-12	C197820-0002	320	24-Oct-12	C198328-0001
265	24-Sep-12	C197312-0003	293	10-Oct-12	C197821-0002	321	24-Oct-12	C198328-0002
266	25-Sep-12	C197442-0002	294	10-Oct-12	C197822-0002	322	24-Oct-12	C198329-0001
267	25-Sep-12	C197443-0002	295	11-Oct-12	C197881-0002	323	24-Oct-12	C198329-0002
268	26-Sep-12	C197478-0002	296	11-Oct-12	C197882-0002	324	24-Oct-12	C198330-0002
269	26-Sep-12	C197479-0002	297	11-Oct-12	C197883-0002	325	24-Oct-12	C198331-0002
270	26-Sep-12	C197480-0002	298	11-Oct-12	C197884-0002	326	24-Oct-12	C198332-0001
271	26-Sep-12	C197481-0002	299	11-Oct-12	C197885-0002	327	24-Oct-12	C198332-0002
272	26-Sep-12	C197482-0002	300	11-Oct-12	C197886-0002	328	24-Oct-12	C198333-0002
273	26-Sep-12	C197483-0002	301	11-Oct-12	C197887-0002	329	25-Oct-12	C198389-0002
274	26-Sep-12	C197484-0002	302	12-Oct-12	C197924-0002	330	26-Oct-12	C198445-0002
275	26-Sep-12	C197485-0002	303	16-Oct-12	C197993-0002	331	30-Oct-12	C198507-0001
276	27-Sep-12	C197565-0002	304	16-Oct-12	C197994-0002	332	30-Oct-12	C198507-0002
277	02-Oct-12	C197645-0001	305	17-Oct-12	C198070-0002	333	31-Oct-12	C198544-0002

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
334	31-Oct-12	C198545-0002	362	08-Nov-12	C198805-0001	390	19-Nov-12	C199037-0002
335	31-Oct-12	C198546-0002	363	08-Nov-12	C198805-0002	391	20-Nov-12	C199066-0001
336	31-Oct-12	C198547-0002	364	08-Nov-12	C198806-0001	392	20-Nov-12	C199066-0002
337	31-Oct-12	C198548-0001	365	08-Nov-12	C198806-0002	393	20-Nov-12	C199067-0002
338	31-Oct-12	C198548-0002	366	08-Nov-12	C198807-0001	394	21-Nov-12	C199135-0002
339	31-Oct-12	C198549-0002	367	08-Nov-12	C198807-0002	395	21-Nov-12	C199136-0002
340	31-Oct-12	C198550-0001	368	14-Nov-12	C198951-0001	396	21-Nov-12	C199137-0002
341	31-Oct-12	C198550-0002	369	14-Nov-12	C198951-0002	397	21-Nov-12	C199138-0001
342	31-Oct-12	C198551-0001	370	14-Nov-12	C198952-0001	398	21-Nov-12	C199138-0002
343	31-Oct-12	C198551-0002	371	14-Nov-12	C198952-0002	399	21-Nov-12	C199140-0002
344	01-Nov-12	C198596-0002	372	14-Nov-12	C198953-0001	400	21-Nov-12	C199141-0001
345	02-Nov-12	C198625-0001	373	14-Nov-12	C198953-0002	401	21-Nov-12	C199141-0002
346	02-Nov-12	C198625-0002	374	14-Nov-12	C198954-0001	402	21-Nov-12	C199142-0001
347	02-Nov-12	C198626-0002	375	14-Nov-12	C198954-0002	403	21-Nov-12	C199142-0002
348	06-Nov-12	C198683-0001	376	14-Nov-12	C198955-0001	404	22-Nov-12	C199164-0002
349	06-Nov-12	C198683-0002	377	14-Nov-12	C198955-0002	405	23-Nov-12	C199215-0001
350	07-Nov-12	C198736-0002	378	14-Nov-12	C198956-0001	406	23-Nov-12	C199215-0002
351	07-Nov-12	C198737-0001	379	14-Nov-12	C198956-0002	407	27-Nov-12	C199277-0001
352	07-Nov-12	C198737-0002	380	15-Nov-12	C198989-0001	408	27-Nov-12	C199277-0002
353	07-Nov-12	C198738-0002	381	15-Nov-12	C198989-0002	409	27-Nov-12	C199278-0001
354	07-Nov-12	C198739-0002	382	15-Nov-12	C198990-0001	410	27-Nov-12	C199278-0002
355	07-Nov-12	C198740-0002	383	15-Nov-12	C198990-0002	411	28-Nov-12	C199348-0002
356	07-Nov-12	C198741-0001	384	15-Nov-12	C198991-0001	412	28-Nov-12	C199349-0002
357	07-Nov-12	C198741-0002	385	15-Nov-12	C198991-0002	413	28-Nov-12	C199350-0002
358	07-Nov-12	C198742-0001	386	16-Nov-12	C199020-0001	414	28-Nov-12	C199351-0002
359	07-Nov-12	C198742-0002	387	16-Nov-12	C199020-0002	415	28-Nov-12	C199352-0001
360	08-Nov-12	C198804-0001	388	16-Nov-12	C199021-0001	416	28-Nov-12	C199352-0002
361	08-Nov-12	C198804-0002	389	16-Nov-12	C199021-0002	417	28-Nov-12	C199353-0001

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
418	28-Nov-12	C199353-0002	420	41241	C199355-0001	422	41242	C199389-0002
419	28-Nov-12	C199354-0002	421	41241	C199355-0002	423	41242	C199391-0002
2012 WD samples tested by only ELISA no LC-MS/MS = 423								

TABLE E17. 2012 DRINKING WATER (WD) SAMPLES TESTED BY LC-MS/MS ONLY FOR MICROCYSTINS

Counter	Date	LIMS #	Counter	Date	LIMS #	Counter	Date	LIMS #
1	02-May-12	C193550-0001	5	03-Jul-12	C195195-0001	9	28-Aug-12	C196749-0003
2	02-May-12	C193550-0002	6	03-Jul-12	C195195-0004	10	28-Aug-12	C196749-0004
3	02-May-12	C193550-0003	7	28-Aug-12	C196749-0001	11	20-Dec-12	C199839-0001
4	02-May-12	C193550-0004	8	28-Aug-12	C196749-0002	12	20-Dec-12	C199839-0002
2012 WD samples tested by LC-MS/MS only =12								

TABLE E18. 2012 DRINKING WATER (WD) SAMPLES TESTED BY BOTH ELISA AND LC-MS/MS

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
1	12-Jan-12	C191512-0001	25	04-Jul-12	C195210-0001	49	09-Aug-12	C196242-0001
2	19-Jan-12	C191643-0001	26	05-Jul-12	C195269-0001	50	09-Aug-12	C196243-0001
3	02-Feb-12	C191888-0001	27	05-Jul-12	C195270-0001	51	09-Aug-12	C196244-0001
4	16-Feb-12	C192116-0001	28	05-Jul-12	C195271-0001	52	09-Aug-12	C196245-0001
5	01-Mar-12	C192364-0001	29	11-Jul-12	C195425-0001	53	09-Aug-12	C196246-0001
6	15-Mar-12	C192647-0001	30	11-Jul-12	C195428-0001	54	09-Aug-12	C196247-0001
7	29-Mar-12	C192912-0001	31	11-Jul-12	C195429-0001	55	10-Aug-12	C196268-0001
8	12-Apr-12	C193110-0001	32	12-Jul-12	C195468-0001	56	14-Aug-12	C196334-0001
9	26-Apr-12	C193451-0001	33	18-Jul-12	C195613-0001	57	15-Aug-12	C196400-0001
10	10-May-12	C193785-0001	34	18-Jul-12	C195615-0001	58	15-Aug-12	C196401-0001
11	24-May-12	C194131-0001	35	18-Jul-12	C195618-0001	59	15-Aug-12	C196402-0001
12	07-Jun-12	C194508-0001	36	18-Jul-12	C195619-0001	60	15-Aug-12	C196403-0001
13	13-Jun-12	C194674-0001	37	25-Jul-12	C195836-0001	61	15-Aug-12	C196404-0001
14	13-Jun-12	C194676-0001	38	25-Jul-12	C195839-0001	62	15-Aug-12	C196405-0001
15	13-Jun-12	C194677-0001	39	25-Jul-12	C195841-0001	63	15-Aug-12	C196406-0001
16	14-Jun-12	C194719-0001	40	01-Aug-12	C196003-0001	64	15-Aug-12	C196407-0001
17	20-Jun-12	C194884-0001	41	01-Aug-12	C196005-0001	65	15-Aug-12	C196408-0001
18	20-Jun-12	C194886-0001	42	01-Aug-12	C196008-0001	66	15-Aug-12	C196409-0001
19	20-Jun-12	C194887-0001	43	01-Aug-12	C196009-0001	67	17-Aug-12	C196495-0001
20	20-Jun-12	C194889-0001	44	08-Aug-12	C196173-0001	68	21-Aug-12	C196542-0001
21	27-Jun-12	C195081-0001	45	08-Aug-12	C196174-0001	69	21-Aug-12	C196543-0001
22	27-Jun-12	C195085-0001	46	09-Aug-12	C196239-0001	70	21-Aug-12	C196544-0001
23	27-Jun-12	C195087-0001	47	09-Aug-12	C196240-0001	71	22-Aug-12	C196608-0001
24	28-Jun-12	C195127-0001	48	09-Aug-12	C196241-0001	72	22-Aug-12	C196609-0001

APPENDIX E

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
73	22-Aug-12	C196610-0001	97	05-Sep-12	C196891-0001	121	19-Sep-12	C197327-0001
74	22-Aug-12	C196613-0001	98	06-Sep-12	C196945-0001	122	19-Sep-12	C197328-0001
75	22-Aug-12	C196614-0001	99	06-Sep-12	C196946-0001	123	19-Sep-12	C197329-0001
76	22-Aug-12	C196615-0001	100	06-Sep-12	C196947-0001	124	19-Sep-12	C197330-0001
77	22-Aug-12	C196616-0001	101	06-Sep-12	C196948-0001	125	19-Sep-12	C197331-0001
78	22-Aug-12	C196617-0001	102	06-Sep-12	C196949-0001	126	19-Sep-12	C197332-0001
79	23-Aug-12	C196637-0001	103	07-Sep-12	C196980-0001	127	19-Sep-12	C197333-0001
80	23-Aug-12	C196652-0001	104	07-Sep-12	C196981-0001	128	19-Sep-12	C197334-0001
81	23-Aug-12	C196652-0002	105	07-Sep-12	C196982-0001	129	20-Sep-12	C197368-0001
82	23-Aug-12	C196652-0003	106	10-Sep-12	C197019-0001	130	20-Sep-12	C197369-0001
83	28-Aug-12	C196730-0001	107	11-Sep-12	C197053-0001	131	25-Sep-12	C197442-0001
84	28-Aug-12	C196731-0001	108	12-Sep-12	C197139-0001	132	25-Sep-12	C197443-0001
85	29-Aug-12	C196767-0001	109	12-Sep-12	C197140-0001	133	26-Sep-12	C197478-0001
86	29-Aug-12	C196768-0001	110	12-Sep-12	C197141-0001	134	26-Sep-12	C197479-0001
87	29-Aug-12	C196769-0001	111	12-Sep-12	C197142-0001	135	26-Sep-12	C197480-0001
88	29-Aug-12	C196770-0001	112	12-Sep-12	C197143-0001	136	26-Sep-12	C197481-0001
89	29-Aug-12	C196771-0001	113	12-Sep-12	C197144-0001	137	26-Sep-12	C197482-0001
90	29-Aug-12	C196772-0001	114	12-Sep-12	C197145-0001	138	26-Sep-12	C197483-0001
91	29-Aug-12	C196773-0001	115	12-Sep-12	C197146-0001	139	26-Sep-12	C197484-0001
92	29-Aug-12	C196774-0001	116	12-Sep-12	C197147-0001	140	26-Sep-12	C197485-0001
93	29-Aug-12	C196775-0001	117	12-Sep-12	C197148-0001	141	27-Sep-12	C197565-0001
94	31-Aug-12	C196869-0001	118	13-Sep-12	C197185-0001	142	02-Oct-12	C197648-0001
95	05-Sep-12	C196889-0001	119	18-Sep-12	C197264-0001	143	02-Oct-12	C197649-0001
96	05-Sep-12	C196890-0001	120	19-Sep-12	C197326-0001	144	03-Oct-12	C197689-0001

	Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS			Samples tested by both ELISA and LC-MS/MS	
Counter	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**	**Counter**	**Date**	**LIMS #**
145	03-Oct-12	C197690-0001	170	17-Oct-12	C198072-0001	195	07-Nov-12	C198739-0001
146	03-Oct-12	C197691-0001	171	17-Oct-12	C198073-0001	196	07-Nov-12	C198740-0001
147	03-Oct-12	C197692-0001	172	17-Oct-12	C198074-0001	197	19-Nov-12	C199037-0001
148	03-Oct-12	C197693-0001	173	17-Oct-12	C198075-0001	198	20-Nov-12	C199067-0001
149	03-Oct-12	C197694-0001	174	17-Oct-12	C198076-0001	199	21-Nov-12	C199135-0001
150	03-Oct-12	C197695-0001	175	18-Oct-12	C198126-0001	200	21-Nov-12	C199136-0001
151	04-Oct-12	C197741-0001	176	18-Oct-12	C198127-0001	201	21-Nov-12	C199137-0001
152	05-Oct-12	C197779-0001	177	19-Oct-12	C198190-0001	202	21-Nov-12	C199139-0001
153	09-Oct-12	C197802-0001	178	23-Oct-12	C198267-0001	203	21-Nov-12	C199140-0001
154	10-Oct-12	C197819-0001	179	24-Oct-12	C198325-0001	204	22-Nov-12	C199164-0001
155	10-Oct-12	C197820-0001	180	24-Oct-12	C198326-0001	205	28-Nov-12	C199348-0001
156	10-Oct-12	C197821-0001	181	24-Oct-12	C198330-0001	206	28-Nov-12	C199349-0001
157	10-Oct-12	C197822-0001	182	24-Oct-12	C198331-0001	207	28-Nov-12	C199350-0001
158	11-Oct-12	C197881-0001	183	24-Oct-12	C198333-0001	208	28-Nov-12	C199351-0001
159	11-Oct-12	C197882-0001	184	25-Oct-12	C198389-0001	209	21-Nov-12	C199136-0001
160	11-Oct-12	C197883-0001	185	26-Oct-12	C198445-0001	210	21-Nov-12	C199137-0001
161	11-Oct-12	C197884-0001	186	31-Oct-12	C198544-0001	211	21-Nov-12	C199139-0001
162	11-Oct-12	C197885-0001	187	31-Oct-12	C198545-0001	212	21-Nov-12	C199140-0001
163	11-Oct-12	C197886-0001	188	31-Oct-12	C198546-0001	213	22-Nov-12	C199164-0001
164	11-Oct-12	C197887-0001	189	31-Oct-12	C198547-0001	214	28-Nov-12	C199348-0001
165	12-Oct-12	C197924-0001	190	31-Oct-12	C198549-0001	215	28-Nov-12	C199349-0001
166	16-Oct-12	C197993-0001	191	01-Nov-12	C198596-0001	216	28-Nov-12	C199350-0001
167	16-Oct-12	C197994-0001	192	02-Nov-12	C198626-0001	217	28-Nov-12	C199351-0001
168	17-Oct-12	C198070-0001	193	07-Nov-12	C198736-0001	218	28-Nov-12	C199354-0001
169	17-Oct-12	C198071-0001	194	07-Nov-12	C198738-0001	219	29-Nov-12	C199391-0001
2012 WD samples tested by both ELISA and LC-MS/MS = 219								

APPENDIX F - GRAPHICAL PRESENTATION OF ELISA AND LC-MS/MS WORKLOAD CONTRIBUTION

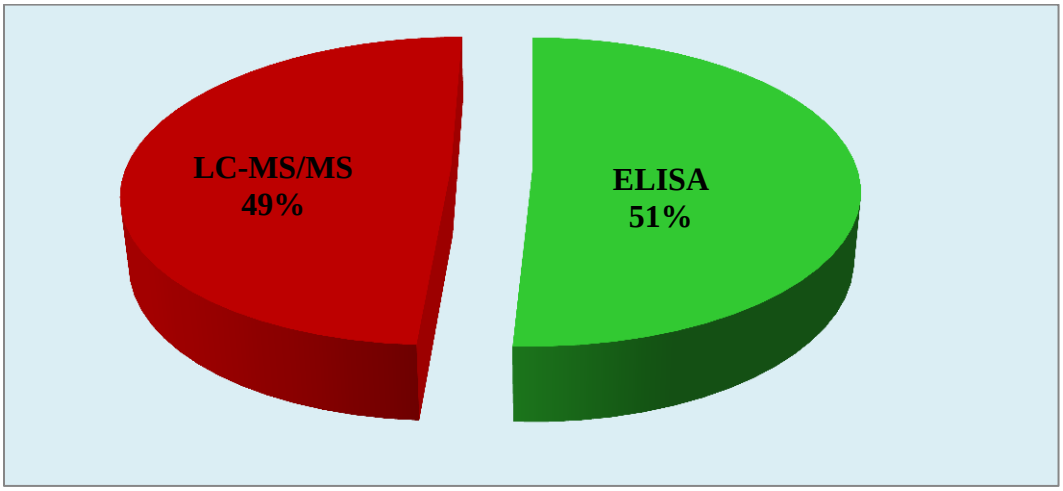

FIGURE F1. 2010 (WS & WD) MICROCYSTINS SAMPLES TESTED BY ELISA & LC-MS/MS

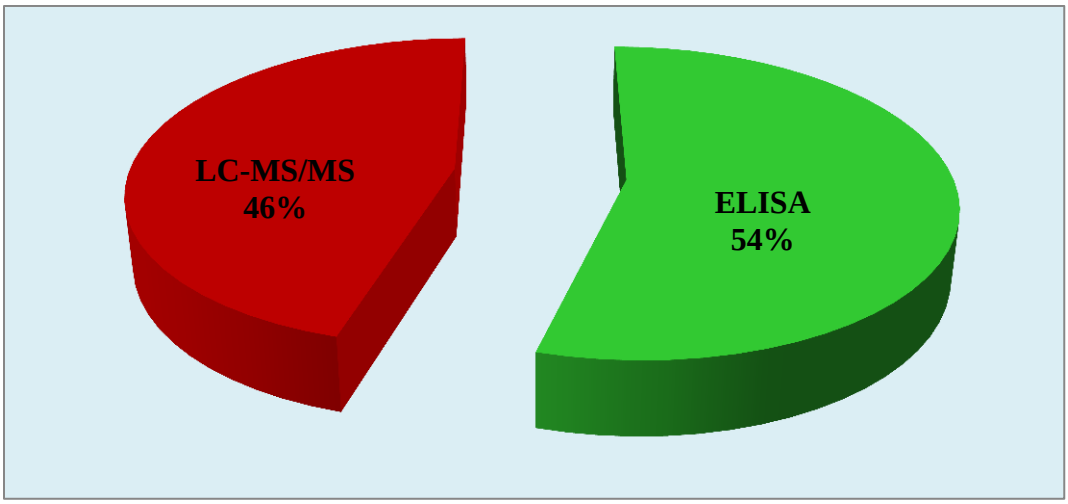

FIGURE F2. 2011 (WS & WD) MICROCYSTINS SAMPLES TESTED BY ELISA & LC-MS/MS

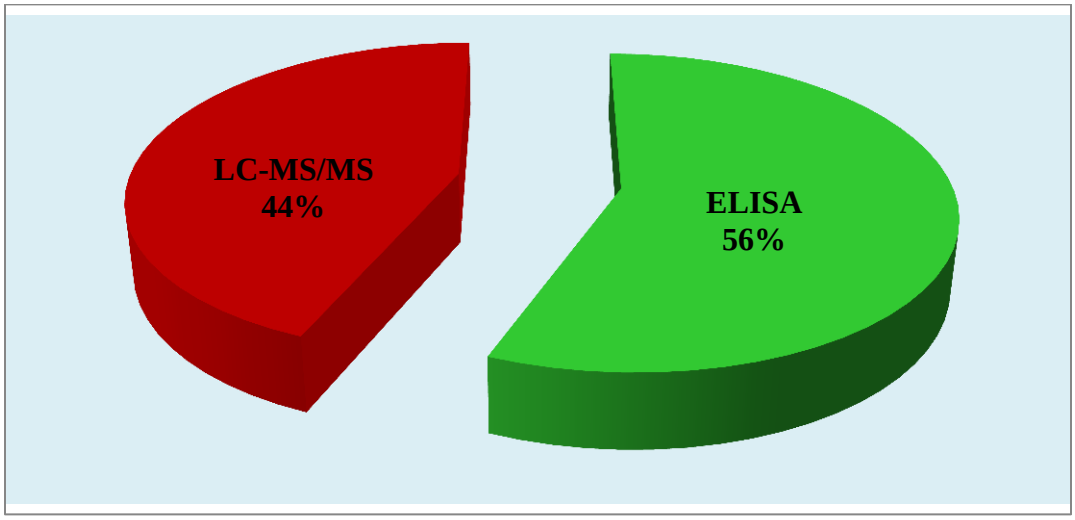

FIGURE F3. 2012 (WS & WD) MICROCYSTINS SAMPLES TESTED BY ELISA & LC-MS/MS

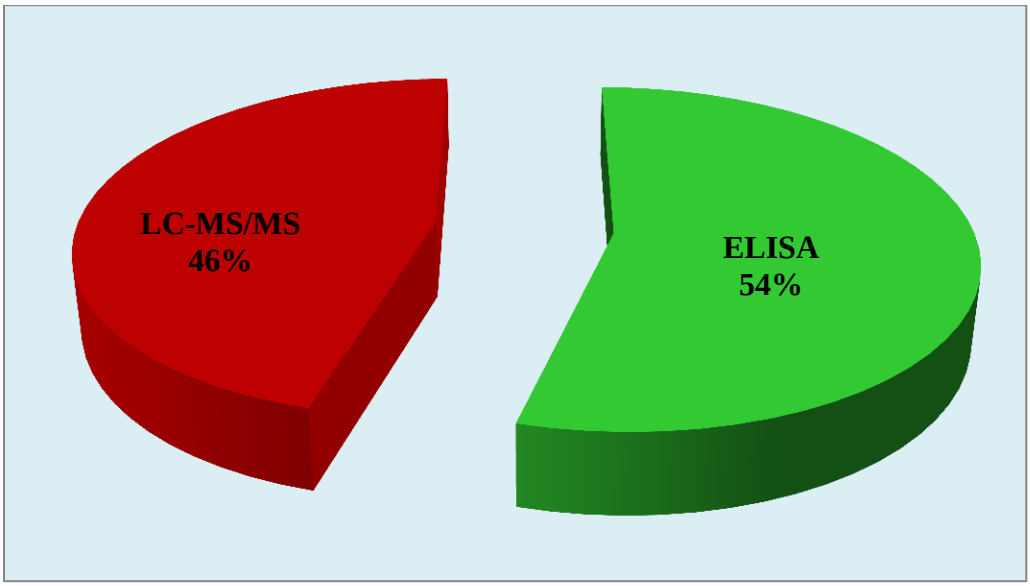

FIGURE F4. 2010 TO 2012 (WS & WD) MICROCYSTINS SAMPLES TESTED BY ELISA & LC-MS/MS

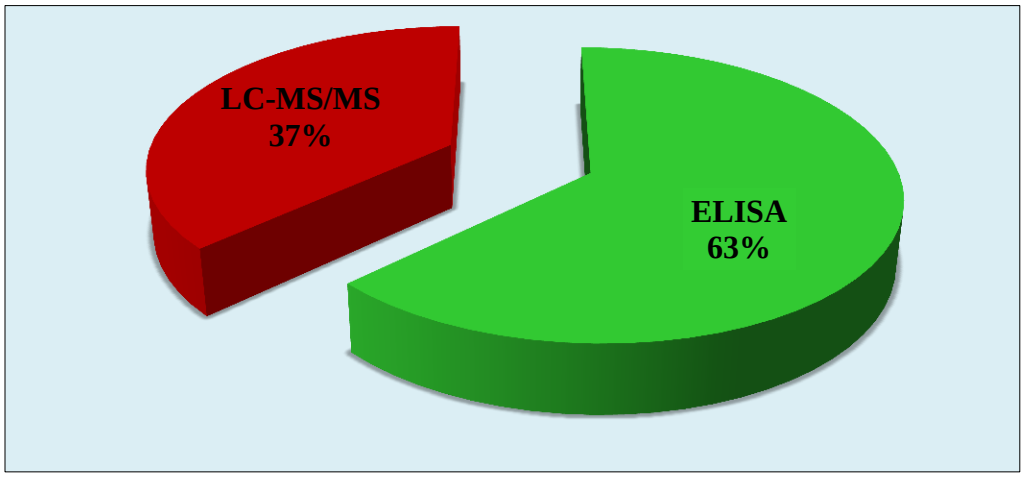

FIGURE F5. 2010 TO 2012 WD MICROCYSTINS SAMPLES TESTED BY ELISA & LC-MS/MS

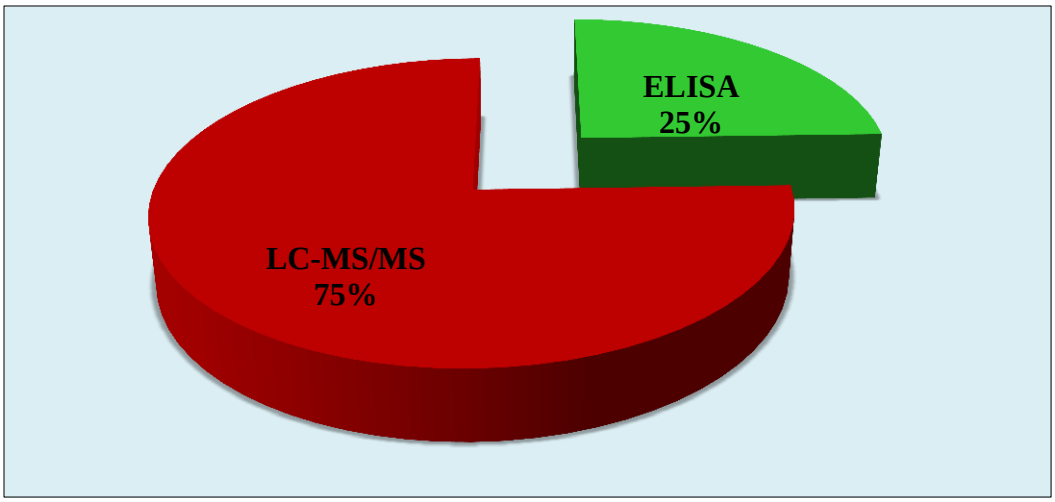

FIGURE F6. 2010 TO 2012 WS MICROCYSTINS SAMPLES TESTED BY ELISA & LC-MS/MS

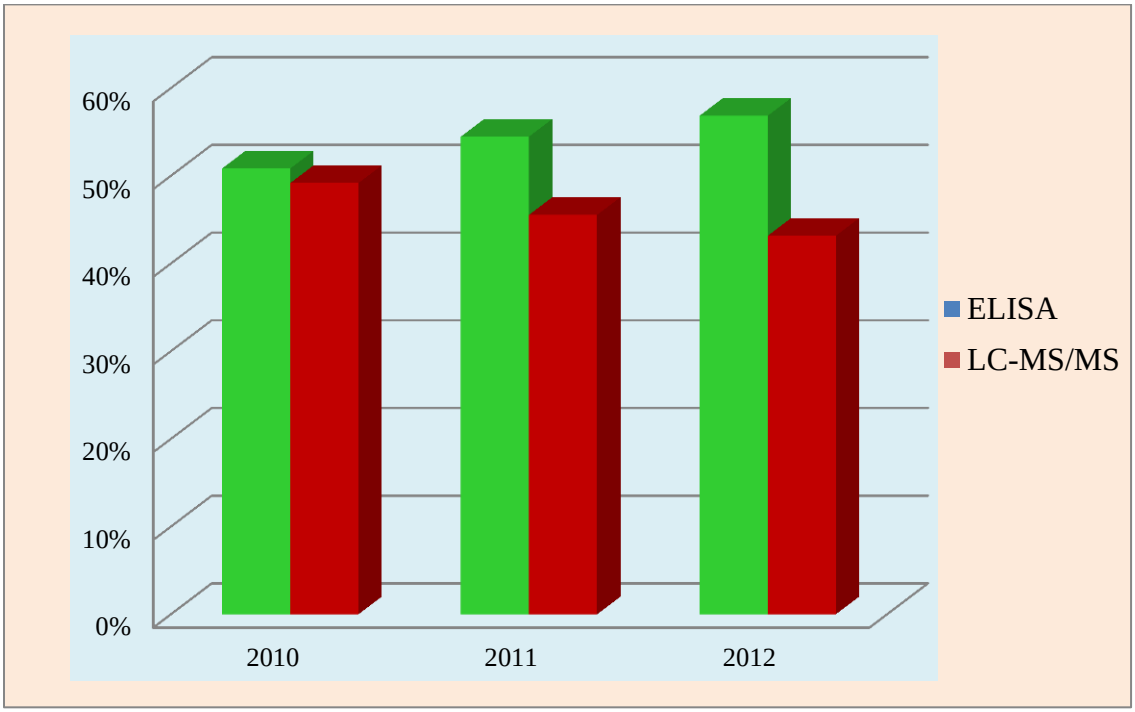

FIGURE F7. 2010 TO 2012 (WS & WD) MICROCYSTINS WORKLOAD RELIEVED BY ELISA & LC-MS/MS

APPENDIX G - ELISA PROCÉDURE FOR THE ANALYSIS OF MICROCYSTINS (ABRAXIS, PRODUCT NO. 520011)

Enzyme-Linked Immunosorbent Assay for the Congener- Independent Determination of microcystins and nodularins in water samples.

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A. General Description

The Abraxis microcystins-ADDA ELISA is an immunoassay for the quantitative and sensitive congener independent* detection of microcystins and nodularins in water samples. No additional sample preparation is required prior to analysis. If necessary, positive samples can be confirmed by HPLC, protein phosphatase assay, or other conventional methods.

B. Test Principle

The test is an indirect competitive ELISA for the congener-independent detection of microcystins and nodularins. It is based on the recognition of microcystins, nodularins, and their congeners by specific antibodies. Toxin, when present in a sample and a microcystins-protein analogue immobilized on the plate competes for the binding sites of the anti-microcystins/Nodularins antibodies in solution. The plate is then washed and a second antibody-HRP label is added. After a second washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Materials

1. Microtiter plate (12 X 8 strips) coated with an analog of microcystins conjugated to a protein.
2. Standards (6) and Control (1): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb; Control at 0.75 ± 0.185 ppb
3. Sample Diluent (for dilution of samples above the range of the curve)
4. Antibody Solution
5. Anti-Sheep-HRP Conjugate Solution

6. Wash Solution (5X) Concentrate, must be diluted prior to use, see Test Preparation (Section D)
7. Substrate (Color) Solution (TMB)
8. Stop Solution
9. Micro-pipettes with disposable plastic tips (20-200 µL)
10. Multi-channel pipette (50-300 µL) or stepper pipette with plastic tips (50-300 µL)
11. Deionized or distilled water
12. Paper towels or equivalent absorbent material
13. Timer
14. Tape or parafilm
15. Microtiter plate reader (wavelength 450 nm)
16. Microtiter plate washer (optional)

C. Sample Collection and Handling

Collect water samples in glass containers and test within 24 hours. If the samples must be held for longer periods (up to 5 days), it should be stored in refrigerator. For storage periods greater than 5 days, samples should be stored frozen. If total microcystins concentration (free and cell bound) is required, an appropriate cell lysing procedure (freeze and thaw, sonication, quick Lyse™, etc.) must be performed prior to analysis.

D. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp 2									
B	Std 0	Std 4	Samp 2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Cont.										
F	Std 2	Cont.										
G	Std 3	Samp 1										
H	Std 3	Samp 1										

Std 0 - Std5: Standard, Cont: Control, Sample 1, Sample 2, etc: Sample

E. Assay Procedure

1. Add 125µL of the standard solutions, control, or samples into the wells of the test strips of blank plate according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 125µL of the antibody solution to the individual wells of blank plate successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the bench top for 30 seconds. Incubate on a shaker for 30 minutes at room temperature.
3. Transfer 100µL of each pre-incubated standard and control, or sample from blank plate to the appropriate wells of the microcystins coated microtiter plate. Incubate the microtiter plate for 90 minutes at room temperature on a shaker.
4. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the 1X wash buffer solution. Use at least a volume of 250 µL of wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add 100 µL of the enzyme conjugate solution to the individual wells successively using a multichannel pipette or a stepping pipette. Cover the wells with Para film or tape and mix the contents by moving the strip holder in a circular motion on the bench top for 30 seconds. Be careful not to spill the contents. Incubate the strips for 30 minutes at room temperature.
6. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the 1X wash buffer solution. Please use at least a volume of 250 µL of wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
7. Add 100 µL of substrate (color) solution to the individual wells successively using a multichannel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from sunlight.
8. Add 50 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.

9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

F. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Log it/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding microcystins concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for the control and samples will then yield levels in ppb of microcystins by interpolation using the standard curve.

Results can also be determined using a spreadsheet macro available from Abraxis upon request. The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of microcystins than standard 1 (0.15 ppb) should be reported as containing < 0.15 ppb of microcystins. Samples showing a higher concentration than standard 5 (5.0 ppb) must be diluted to obtain accurate results. The concentration of the positive control provided should be 0.75 ± 0.185 ppb.

The detection limit for this assay, based on MC-LR, is 0.10 ppb (µg/L). Coefficients of variation (CVs) for standards: <10%; for samples: <15%. *WEB: www.abraxiskits.com*

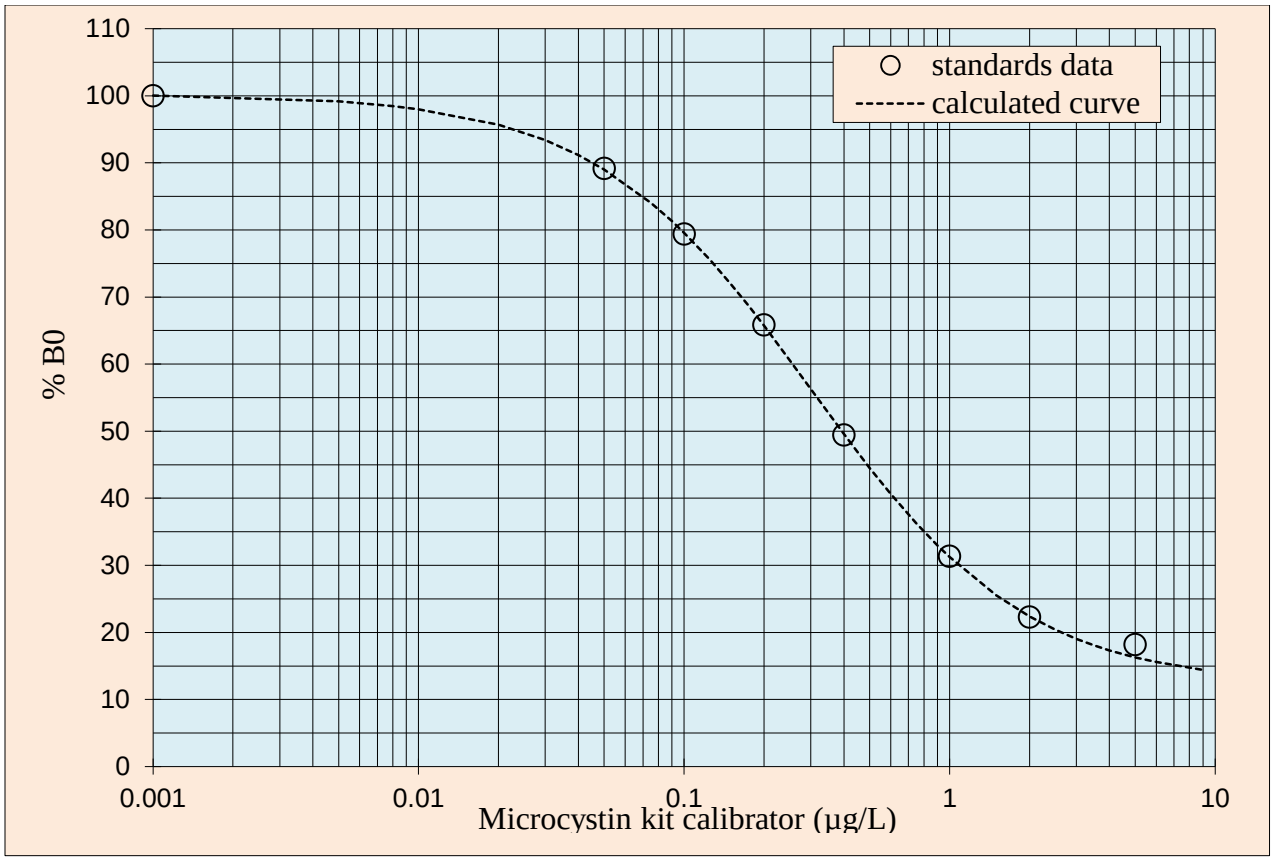


FIG G1. STANDARD CURVE FOR THE CALCULATION OF MICROCYSTINS

APPENDIX H – PP2A PROCEDURE FOR THE ANALYSIS OF MICROCYSTINS (ABRAXIS, PRODUCT NO. 520032)

Microcystins/nodularins PP2A is the test for the detection of microcystins and nodularins in water.

A. General Description

Microcystins/nodularins PP2A Kit is an enzymatic test for the detection of microcystins and nodularins in water. A simple and rapid method that allows to quantify whether the toxin concentration is over the maximum allowed levels (1.0 µg/L, OMS 1998).

B. Test Principle

Microcystins/nodularins PP2A Kit is based on the phosphatase activity inhibition by microcystins. Under normal conditions the phosphatase is able to hydrolyse a specific substrate that can be detected at 405 nm. Samples containing microcystins will inhibit the enzyme activity proportionally to the amount of toxin contained in the sample. The concentration of the toxin in the sample can be calculated using a standard curve.

C. Calculations and Graphic Representation of Results

1. Obtain a standard curve by plotting standards absorbance at 405 nm in the *y axis* and concentration of microcystin-LR in a logarithmic *x axis*. Draw a standard curve. An example of standard curve is shown below:

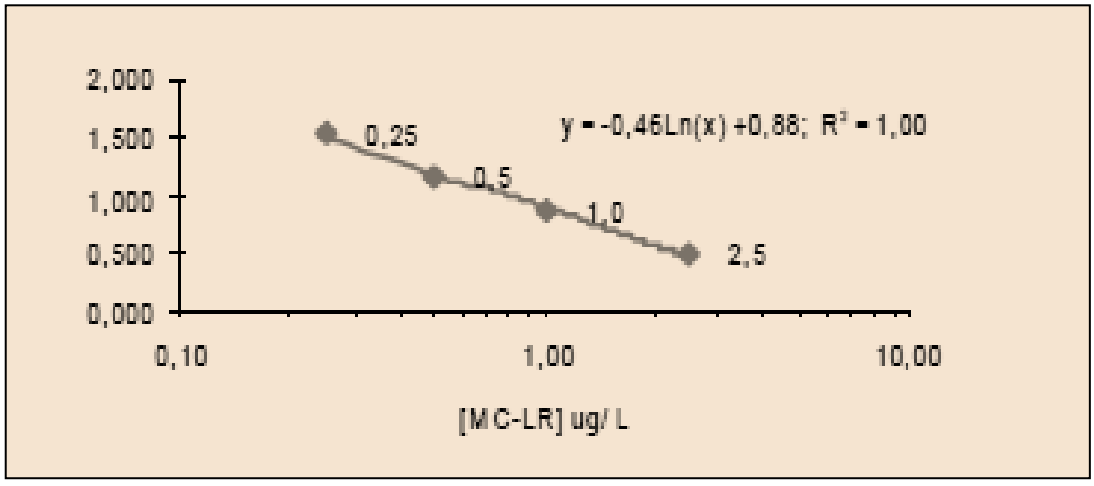


FIG. H1. STANDARD CURVE FOR PP2A

2. The concentration of microcystins in the sample is calculated by interpolating the calibration curve or using the following equation:

$$y = a\ Lnx + b\ x = EXP\ (y\text{-}b/a)$$

Where "***x***" value is concentration of microcystin-LR equivalents in the sample and the "***y***" the absorbance at 405 nm.

D. Sample Preparation

Drinking water (water treated in drinking water stations). Sample preparation is not required.

1. Water from reservoirs, rivers, etc

The content of dissolved microcystins, intracellular microcystins and total microcystins can be determined by following the scheme in Fig H2.

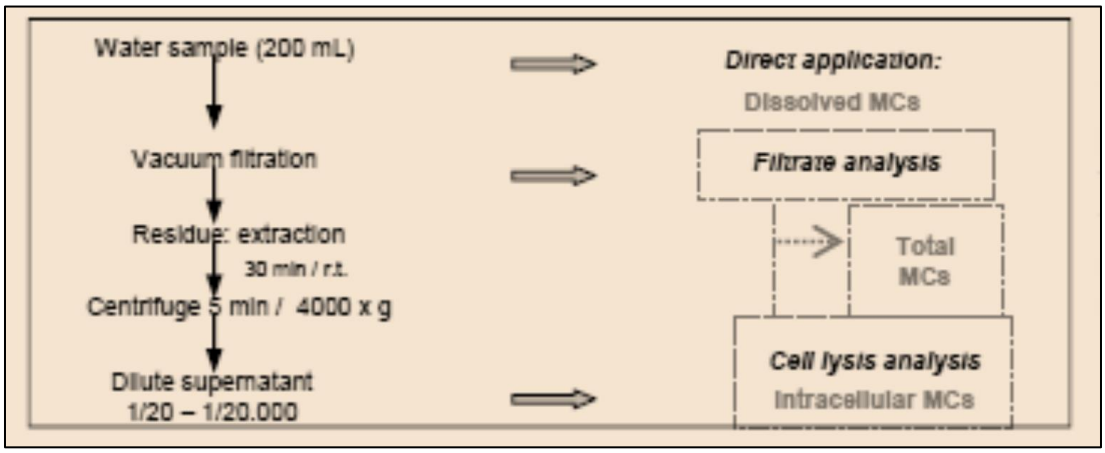


FIG.H2. SCHEME PROCEDURE FOR WATER SAMPLES FROM RESERVOIRS, RIVERS, ETC.

1.1 Dissolved microcystins: Sample preparation is not required. Following the procedure described in Fig.H2**,** dissolved microcystins content will be determined.

1.2 Intracellular microcystins:

a. Take 200 mL of sample and filer in vacuum through a 0.8 µg/L nylon membrane (i.e. Whatman Nylon Membrane Filters, ref.: 7408-004). Reserve the filtrate for further determination of total microcystins.

b. Take the membrane with the residue and place in a glass flask. The membrane can be cut into pieces to improve the extraction step.
c. Add 10 mL of 80% MeOH in water with 0.1% TFA and 0.1% Tween 20. Incubate at room temperature for 30 minutes with gentle stirring and in absence of light.
d. Centrifuge at 4000 g for 5 min.
e. Take the supernatant and dilute it 20 times (dilution 1/20) with distilled water. At this point, the sample is ready to continue the assay as is shown in Section C. This way, the content of Intracellular microcystins is determined. If the concentration of MC-LR equivalents exceeds 2.5µg/L, the assay should be performed with on a range of supernatant dilutions of 1/20, 1/200, 1/2000,

1.3 Total microcystins:

Use the filtrate and perform the assay described in Fig H2. Total microcystins contained in the sample are calculated by adding the concentration of microcystins found in the
filtrate plus the intra cellular microcystins.

NOTE: Presence of thiosulphate (or strong oxidizing reagents) may interfere in the assay, therefore collecting samples in bottles with this chemical or adding it to the simple prior to testing should be avoided.

E Materials

1. Microtiter plate
2. Vials of Phosphatase
3. Standards Microcystins (4): 0.25, 0.50, 1.00, 2.50 ppb
4. 1 vial Chromogenic Substrate
5. 1 vial Phosphatase Dilution Buffer
6. 1 vial Stop Soultion
7. Micro-pipettes with disposable plastic tips (10-200 and 200-1000µL)
8. Multi-channel pipette (50-250µL) or stepper pipette with plastic tips (10-250µL)
9. Microtiter plate reader (wave length 405 nm)
10. Timer

11. Tape or Parafilm
12. Glass vials with Teflon-lined caps
13. Distilled or deionized water
14. Vortex mixer

F Assay Procedure

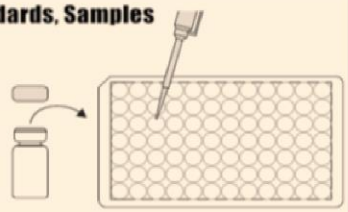
1. Add 50 µg/L of each Microcystin-LR standard in duplicate (i. e.: wells A1 and A2, 0.25 µg/L; wells B1and B2, 0.50 µg/L; wells C1 and C2, 1.00 µg/L; wells D1 and D2, 2.50 µg/L). Using of duplicates or triplicates is recommended.
2. Add 50 µg/L of each sample in duplicate into the remaining wells of the microtiter plate.
3. Add 70 µg/L of the Phosphatase Solution to each well.
4. Add 90 µg/L of Chromogenic Substrate to each well and mix gently. The substrate contains solid in suspension. Do not mix the reagent prior to use and avoid taking any solid.
5. Put the adhesive film on wells and Incubate the plate for 30 minutes at 37 ºC.
6. Add 70 µg/L of Stop Solution to each well. Mix gently.
7. Read the absorbance of samples and standards at 405 nm. Use an empty well as blank, if necessary.

WEB: www.abraxiskits.com

Microcystin PP2A Plate Kit, Concise Procedure

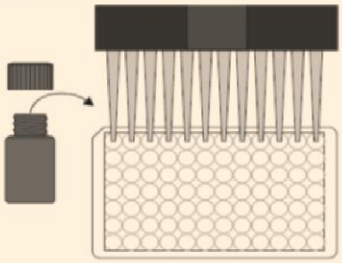
1. Addition of Standards, Samples

Add 50 uL of standard solutions, and samples.



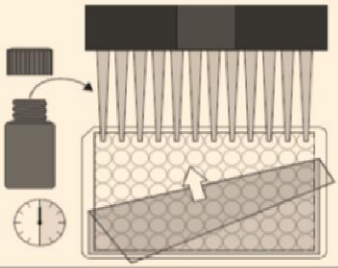
2. Addition of Phosphatase Solution

Add 70 uL of the Phosphatase solution.



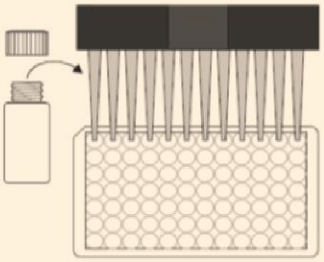
3. Addition of Chromogenic Substrate

Add 90 uL of Chromogenic Substrate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



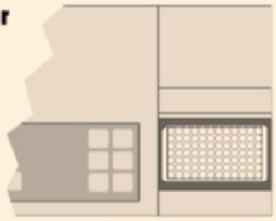
4. Addition of Stopping Solution

Add 70 uL of Stopping Solution.



5. Measurement of Color

Read the absorbance at 405 nm using a microplate ELISA reader. Calculate results.



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APPENDIX I - LC-MS/MS –PROCEDURE OF MICROCYSTINS ANALYSIS AT LASB OF MOE

Principle of the Method

This method is designed to identify and quantify total (free + intracellular) microcystins and anatoxin-a in water by isolation on octadecyl-functionalised (C18) silica gel and analysis by liquid chromatography-(electrospray ionisation) tandem mass spectrometry [LC-(ESI) MS/MS]. Microcystins -LR, -RR, -LA, -YR, -LY, -WR, -HtyR, -HilR, -LW, -LF, desmethylmicrocystins-LR, desmethylmicrocystins-RR and and anatoxin-a are determined quantitatively by multi-point calibration. Nodularin is used as the internal standard.

1. **Filtration**

Samples, method blanks and QC samples are filtered through 47mm GF/C filters using a vacuum filtration funnel. The filtrate is saved in a 1L amber bottle

2. **Freeze drying**

GF/C filters are rolled up and transferred to a polypropylene cryovials. These filter/cryovials are frozen at -40ºC for 30min and then dried for 2 hr. (24 hrs. may be needed for bulk bloom samples). The cells are lysed by the freeze drying process and intracellular toxin is released.

3. Intracellular toxins extraction

Reagent solution of (75% CH_3OH / 25% H_2O) + 0.3% TDFHA (2.2.7) is added to the cryovial. Filtrate from the 1L bottle and the extract (above solution) are sonicated together for 10 min. Then the extract is poured to the corresponding 1 L bottle of filtrate. This procedure is repeated 4 times.

4. Adjustment of pH

The pH of sample (extract+filtrate) is adjusted to 9.5- 10 using a borax buffer solution.

5. Addition of Silica Gel

The internal standard (nodularin) is added to the sample followed by 1.5 g of C18 silica gel and samples are rolled for one hour on a roller apparatus.

6. Vacuum Filtration

The sample is filtered to isolate the C18 silica gel containing the adsorbed target compounds with vacuum filtration using whatman paper 4 and dried again under low flow nitrogen in a drying chamber for 1-3 hrs.

7. Extraction

The target compounds are desorbed with methanol again.

8. Turbo Evaporation

The methanol extract is centrifuged at 1000 rpm for 10 min, filtered and then left on Turbo Vap LV 40 0c to dry. Samples can be left overnight on the turbo bath. Samples are removed from the rack and redissolved in a reagent solution (50%ch3oh/50%h2o). Samples are centrifuged again and filtrate is transferred into glass vials for LC-MS/MS.

9. **LC-MS/MS**

The samples are analysed using liquid chromatography (LC)-multiple reactions monitoring (MRM) tandem mass spectrometry (MS/MS) on a triple quadrupole mass spectrometer.

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