## Supporting Information (SI)

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8 pages, 2 Tables, 2 Figures

**Table S1.** Evaluation of treatment processes for the removal of intracellular and extracellular MCYST-LRs in drinking water supplies.

| Treatment process | Removal efficiency | | Treatment condition | | | Analytical method | Ref |
|---|---|---|---|---|---|---|---|
| | Cyanobacterial cell (intracellular MCYST-LR) | Extracellular MCYST-LR | Initial concentration | Dose | Reaction time | | |
| **Adsorption** | | | | | | | |
| Powdered activated carbon (PAC) | NA[a] | 90% | 10 μg/L | 7.9 mg/L | 7 days | ELISA | 1 |
| Granular activated carbon (GAC) | >60% | 70% | 5 μg/L | N.A. | 6 months | LC/PDA[b] | 2 |
| **Oxidation** | | | | | | | |
| Chlorination | NA | 98% | 10 μg/L | 25.9 mg/L min | | LC/PDA | 3 |
| Ozonation | 91% | 96% | 15 μg/L | 5 mg/L | 60 min | LC/PDA | 4 |
| UV radiation | 71% | NA | 24.6 pg cell | 600 mJ/cm$^2$ | 6 days | ELISA | 5 |
| **Exclusion** | | | | | | | |
| Ultrafiltration (UF) | 98% | NA | 1.5 μg/L | 100 kDa[c] | 4 hr | LC/MS/MS | 6 |
| Nanofiltration (NF) | >99% | >96% | 5.5 μg/L | 200 kDa | 2 days | LC/MS/MS | 6 |
| Reverse osmosis (RO) | >99% | >99% | 70-130 μg/L | 100 Da | 2 days | RPLC[d] | 7 |

[a] NA, not available

[b] LC/PDA, liquid chromatography/photodiode array

[c] Value is based on molecular weight cutoff (MWCO), i.e., refers to the lowest molecular weight solute (in Daltons) in which 90% of the solute is retained by the membrane.

[d] RPLC, reverse-phase liquid chromatography

**Table S2.** Currently available techniques for MCYST-LR analysis

| Detection technique | Biorecognition | Transducer | Read-out | Detection limit | Dynamic range | Whole Analysis time[a] | Ref |
|---|---|---|---|---|---|---|---|
| **Physicochemical analysis** | | | | | | | |
| HPLC-PDA | | | | 10 µg/L | NA | NA | 8 |
| HPLC-MS | | | | 2.6 ng/L | 10–1000 µg/L | | 9 |
| **Biochemical assays** | | | | | | | |
| Protein phosphatase inhibition assay (PPI) | | protein phosphatase 2A | Optical (absorbance) | NA | 0.2–1 µg/L | 100 min | 10 |
| **Molecular analysis** | | | | | | | |
| Real-time PCR | MCYST-LR synthetase gene | SYBR Green | Optical (fluorescence) | 8.8 cells | NA | 100 min | 11 |
| **Immunological assays** | | | | | | | |
| Enzyme-linked immunosorbent assay (ELISA) | | | | µg/L | | | |
| | Indirect competitive | Horseradish peroxidase (HRP) | Optical (absorbance) | 0.004 | 0.004-0.8 | 180 min | 12 |
| | Indirect competitive | HRP | Optical (chemi-luminescence) | 0.2 | 0.2-1.0 | 45 min | 13 |
| Commercial ELISA kit | | | | | | 120 min | |
| Abraxis (MCYST-LR-DM ELISA Kit) | Indirect competitive | HRP | Optical (absorbance) | 0.10 | 0.15-5.0 | | |
| EnviroLogix (QualiPlate™Kit For MCYST-LR) | Indirect competitive | HRP | Optical (absorbance) | 0.15 | 0.16-2.5 | | |
| Strategic diagnostics (EnviroGard® MCYST-LR Plate Kit) | Indirect competitive | NA | Optical (absorbance) | 0.05 | 0.1-1.5 | | |

[a] The overall analysis time per one sample was approximately calculated by the sum of the biological recognition and transduction step, in which neither the purification or concentration of MCYST-LR were considered. In the case of RT-PCR, however, the DNA extraction step was taken into account for estimating the assay time.

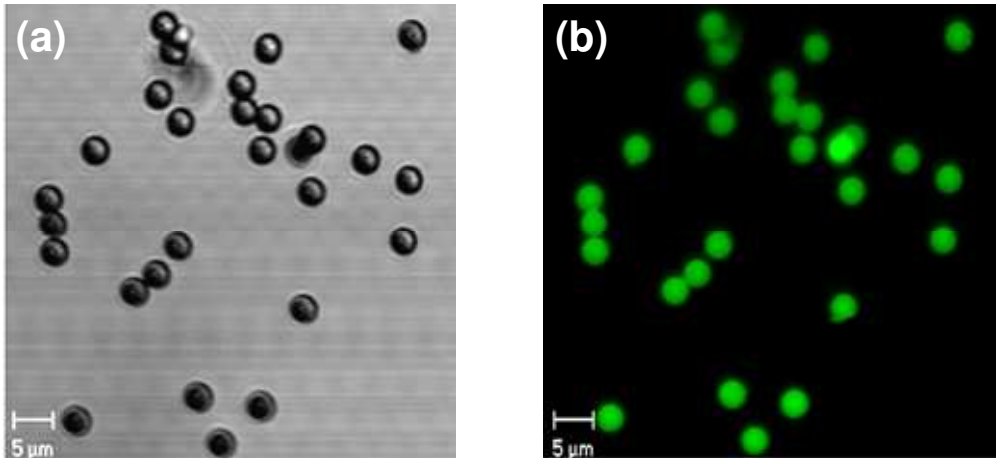


**Figure S1.** Confocal laser scanning microscopic image for visual validation of QD detection probe binding to MB competitor: (a) bare MB competitor, and (b) QD detection probe-conjugated MB competitor.

**I-1. Preparation of QD Detection Probe.**

Monoclonal antibodies (mAbs) against MCYST-LR (MC10E7, Enzo® Life Sciences) were coated on the surface of QDs (Qdot525 antibody conjugation kit, Invitrogen) in accordance with the manufacturer's protocol, with the following minor modifications. 1) 112 μL of 4 μM QDs were activated for 1 h using a 14 μL aliquot of 10 mM hetero-bifunctional crosslinker, 4-(maleimidomethyl)-cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC), which yielded a maleimide functionalized nanocrystal surface. 2) 400 μL of 1 mg/mL Abs were reduced for 30 min using 6.1 μL of 1 M dithiothreitol (DTT) to introduce free sulfhydryls. 3) Excess salts were then removed separately from the QD or Ab solutions via a NAP-5 column to form an efficient linkage between the two components. 4) The reduced Abs were coated onto the activated nanocrystal surface for 1 h at room temperature. 5) The conjugation reaction was quenched for 30 min using 10 μL of 10 mM β-mercaptoethanol, which reacts with any remaining maleimide functional groups on the QDs and converts them to non-reactive derivatives. 6) The Abs-conjugated QDs suspension was then purified and concentrated by multiple round centrifugal washings using a tube filter (100 kD molecular weight cut-off, MWCO) at 4000 rpm for 10 min to remove excess Ab cleaves and salts. 7) The final complex was resuspended and kept in 0.02% sodium azide/PBS as a storage buffer at 4 $^{o}$C prior to use. 8) The conjugate concentration was determined by measuring its optical density at 510 nm the specified wavelength and the using the formula $A=\varepsilon CL$, where A is the absorbance, ε is the molar extinction coefficient (200,000 $M^{-1}$ $cm^{-1}$), C is the molar concentration, and L is the pathlength.

**SI-2. Analysis of Standard MCYST-LR by High-performance Liquid Chromatography/Tandem Electrospray Ionization Mass Spectroscopy (LC-ESI- MS).**

The LC/MS experiments were performed using a Waters Alliance 2695 HPLC system attached to a Micromass Quattro Micro triple quadrupole mass spectrometer coupled to an electrospray.[14] Separation of MCYST-LR samples was performed on a SunFire C18 (2.1 mm × 1.5 mm, i.d. 3.5 μm, Waters). Mobile phase solutions were 0.1 % formic acid (solvent A) and 100% acteonitrile (solvent B), where the gradient elution was 75 % A to 30 % A in 4 min and 30 % A to 75 % A in 6 min. The capillary voltage was set at 3.5 kV, and the cone voltage was 50 V. The desolvation $N_2$ gas temperature was 350 $^oC$, and the flow-rate was 0.3 mL/min; the ion source temperature was set at 150 $^oC$. In addition, the equipment was operated in positive ion mode. Finally, the parent ion of MCYST-LR was recorded at m/z 995.5, a single charged protonated molecular ion, which is then utilized for further quantitative analyses.

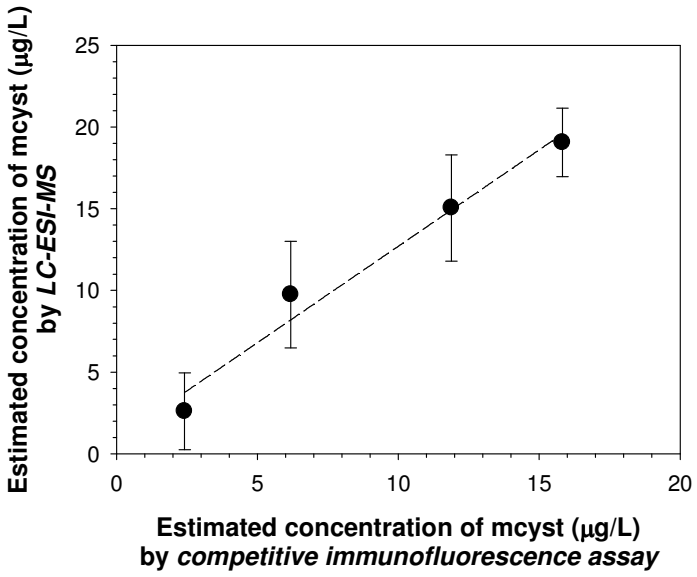


**Figure S2.** Correlation study of the proposed assay with LC-ESI-MS as a reference method ($R^2$=0.974, n=3).

**SI-3. Cultivation of *Microcystis aeruginosa* and Intracellular or Extracellular MCYST Isolation.**

*Microcystis aeruginosa* (NIER 100038) was obtained from the National Institute of Environmental Research (NIER) in Korea. The microorganism was grown in BG 11 medium (Sigma) with continuous illumination at 25 $^{o}$C and constant shaking at 100 rpm. The cyanobacterial batch cultures were separated by filtration through a 0.45 μm membrane filter (Advantec) to prepare the extract samples of extracellular MCYST in the filtrate and intracellular MCYST in the residue.

For isolation of intracellular toxins from the cyanobacterial cells, the resuspended residue of the batch cultures were first lyophilized (-50 $^{o}$C, 0.933 Pa) and the dry biomass weight was measured. Cells were then broken by sonication for 15 min (amplitude 60%, pulse on 5 s, and pulse off 10 s) after being vortexed in methanol 75% (v/v) for 3 min. Supernatant liquid containing MCYST was then separated from cellular debris and unbroken cells after being centrifuged at 10,000 rpm for 10 min, and the resulting supernatant was diluted to less than 10% methanol for further purification steps.

Next, MCYSTs in either extracellular or intracellular extracts were purified and concentrated using a Sep-Pak® Vac 6 cc (1 g) certified tC18 cartridge (Waters) solid-phase extraction that involved activation of a silica cartridge by 10 mL of 100% methanol, equilibration with 10 mL of deionized water, percolation of the sample, and elution of the toxins with 8 mL of 75% methanol, after washing with 10 mL of deionized water and 10 mL of 20% methanol. The isolated extracellular and intracellular MCYSTs were then stored at -20 $^{o}$C under nitrogen gas before analysis.

**REFERENCES**

(1) Mohamed, Z. A.; Carmichael, W. W.; An, J.; El-Sharouny, H. M., Activated carbon removal efficiency of microcystins in an aqueous cell extract of Microcystis aeruginosa and Oscillatoria tenuis strains isolated from Egyptian freshwaters. *Environ. Toxicol.* **1999,** *14* (1), 197−201.

(2) Wang, H.; Ho, L.; Lewis, D. M.; Brookes, J. D.; Newcombe, G., Discriminating and assessing adsorption and biodegradation removal mechanisms during granular activated carbon filtration of microcystin toxins. *Water Res.* **2007,** *41* (18), 4262−4270.

(3) Daly, R. I.; Ho, L.; Brookes, J. D., Effect of Chlorination on Microcystis aeruginosa Cell Integrity and Subsequent Microcystin Release and Degradation. *Environ. Sci. Technol.* **2007,** *41* (12), 4447−4453.

(4) Miao, H.; Tao, W., The mechanisms of ozonation on cyanobacteria and its toxins removal. *Sep. Purif. Technol.* **2009,** *66* (1), 187−193.

(5) Sakai, H.; Oguma, K.; Katayama, H.; Ohgaki, S., Effects of low or medium-pressure UV irradiation on the release of intracellular microcystin. *Water Res.* **2007,** *41* (15), 3458−3464.

(6) Gijsbertsen-Abrahamse, A. J.; Schmidt, W.; Chorus, I.; Heijman, S. G. J., Removal of cyanotoxins by ultrafiltration and nanofiltration. *J. Membrane Sci.* **2006,** *276* (1-2), 252−259.

(7) Neumann, U.; Weckesser, J., Elimination of microcystin peptide toxins from water by reverse osmosis. *Environ. Toxic. Water* **1998,** *13* (2), 143−148.

(8) Kaya, K.; Sano, T.; Inoue, H.; Takagi, H., Selective determination of total normal microcystin by colorimetry, LC/UV detection and/or LC/MS. *Anal. Chimi. Acta* **2001,** *450* (1-2), 73−80.

(9) Zhang, L.; Ping, X.; Yang, Z., Determination of microcystin-LR in surface water using high-performance liquid chromatography/tandem electrospray ionization mass detector.

*Talanta* **2004,** *62* (1), 191−198.

(10) Heresztyn, T.; Nicholson, B. C., Determination of cyanobacterial hepatotoxins directly in water using a protein phosphatase inhibition assay. *Water Res.* **2001,** *35* (13), 3049−3056.

(11) Furukawa, K.; Noda, N.; Tsuneda, S.; Saito, T.; Itayama, T.; Inamori, Y., Highly sensitive real-time PCR assay for quantification of toxic cyanobacteria based on microcystin synthetase a gene. *J. Biosci. Bioeng.* **2006,** *102* (2), 90−96.

(12) Lindner, P.; Molz, R.; Yacoub-George, E.; Dürkop, A.; Wolf, H., Development of a highly sensitive inhibition immunoassay for microcystin-LR. *Anal. Chim. Acta* **2004,** *521* (1), 37−44.

(13) Lindner, P.; Molz, R.; Yacoub-George, E.; Wolf, H., Rapid chemiluminescence biosensing of microcystin-LR. *Anal. Chim. Acta* **2009,** *636* (2), 218−223.

(14) Kim, I. S.; Nguyen, G. H.; Kim, S. Y.; Lee, J. W.; Yu, H. W., Evaluation of methods for cyanobacterial cell lysis and toxin (microcystin-LR) extraction using chromatographic and mass spectrometric analyses. *Environ. Eng. Res.* **2009**, *14* (4), 250−254.