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ITREOH Building of Regional Capacity to Monitor Recreational Water:

Development of a Non-commercial Microcystin ELISA and Its Impact on Public Health Policy

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In 2001, a University of California, Davis–University of the Republic, Montevideo, partnership created a Fogarty ITREOH program to exploit the potential of ELISA to provide a low-cost environmental analysis attractive to economically distressed countries of temperate South America. This paper describes the development and validation of an ELISA method for the determination of *Cyanobacteria* microcystin toxins in algal blooms, which release hepatotoxic metabolites that can reach toxic levels in rivers, lakes, or coastal estuaries used for recreation or water supplies. The assay made possible the first systematic monitoring of water from the Rio de la Plata at Montevideo over two summers. The project has been integrated into a bi-national effort to monitor the Rio de la Plata. *Key words:* ELISA; microcystins; cyanobacteria; Fogarty International Center; Uruguay, Rio de la Plata; water pollution; monitoring.

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Cyanobacteria, or blue-green algae, can produce dense cellular growth called blooms in nutrient-rich fresh water such as eutrophic lakes and rivers contaminated by anthropogenic sources such as sewage effluent and agricultural runoff.¹ Some of the

Cyanobacteria species produce hepatotoxins, neurotoxins, cytotoxins, or dermatotoxins, which can pose a public health risk to humans and animals that drink this water or use it for recreational purposes. The most common of these cyanobacterial toxins are the microcystins, hepatotoxins produced by the genera *Microcystis*, *Anabaena*, and *Plankthotrix*, which are structurally related cyclic heptapeptides that contain several D-amino acids, N-methyldehydroalanine, and an unusual 20-carbon amino acid, 3-amino-9-methoxy-2-6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda). The relevance of cyanobacterial toxins such as the microcystins to human health is underscored by a tragedy that occurred in a hemodialysis clinic in Brazil in 1996, where over 100 patients became ill and developed acute liver failure, and more than 50 died, as a result of microcystin intoxication from contaminated water.² Cyanobacterial toxins can also cause severe effects on targets other than humans, including acute large-scale fish kills and subtler effects such as decreased food sources for fish, for example zooplankton. The World Health Organization has proposed a provisional public health guideline of less than 1 µg/L of microcystin-LR equivalents in drinking water.³ Bioaccumulation of microcystins in fish has been reported, so exposure to contaminated food is another major public health concern.^{4,5} Thus, sensitive and robust analytical methods are required for detection of microcystins in waters at risk of algal blooms. ELISA diagnostic kits for microcystins are commercially available, and have been used for analysis of toxins in water.⁶

Cyanobacterial blooms and scums have been reported to occur commonly in lakes, ponds, and reservoirs in Argentina,^{7,8} Brazil,^{9,10} and Chile,^{11,12} where a major industry—fish farming—is threatened, and in Uruguay, including the Rio de la Plata.¹³ Over the last few years, blooms of *Cyanobacteria* have been an increasing phenomenon in the waters of the Rio de la Plata, with unforeseen intensity after the summer of 2001.¹⁴ To characterize the potential hazards associated with the different uses of water, it is important to carry out adequate monitoring programs, including deter-

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TABLE 1 WHO Guidelines for the Safe Management of Recreational Waters*

Guidance Level or Situation	Health Risks
Relatively low probability of adverse health effects 20,000 cyanobacterial cells /mL or 10 µg chlorophyll a/L 2–4 mg microcystin/L (up to 10 mg/L for very toxic blooms)	Short-term adverse outcomes, e.g., skin irritations, gastrointestinal illness
Moderate probability of adverse health effects 100,000 cyanobacterial cells/mL or 50 µg/L chlorophyll a 20 µg microcystin /L (up to 50 µg/L for very toxic blooms)	Potential for long-term illness Short-term adverse health outcomes
High probability of adverse health effects Cyanobacterial scum formation	Potential for acute poisoning Potential for long-term illness Short-term adverse health outcomes

Source: Chorus and Bartram.¹⁵

minations of chlorophyll a (an indicator of phytoplankton biomass), salinity, and surface current circulation, in order to evaluate transport of harmful algae, as well as other factors involved, such as the temporal and geographic distribution of inorganic nutrients and other phytoplankton growth factors. WHO experts have proposed a classification of the risk associated with recreational activities¹⁵ based on the chlorophyll a content, the number of cyanobacterial cells, and the different ranges of toxin contents associated with these parameters as calculated from the average toxin contents of the most common species (Table 1).

In most South American countries, the lack of financial resources for environmental monitoring programs restricts the information available to inform governmental decision makers to set rational public policies. The Rio de la Plata, which drains the second largest watershed in South America after the Amazon river, is a tidal river in the upper (western) part and an estuary in the areas near Montevideo.¹⁶ The numerous dams built in the Rio de la Plata basin, excessive use of fertilizers, and inadequate treatment of sewage in the whole region make the river and the nearby ocean

water rich in nitrogen and phosphorus,¹⁷ giving rise to ideal conditions for encouraging algal and Cyanobacteria blooms.

The coast of the Rio de la Plata, including Montevideo, the capital of Uruguay and a city with a population of about 1.5 million, has an extensive network of beaches directly on the river. The recreational use of all of these beaches for swimming, boating, and fishing during the summer and warmer months has major economic and social implications for the local population and their government agencies. The health risks associated with the use of these beaches for recreational purposes have remained unknown, due to the difficulties of developing cost-effective methods for monitoring cyanotoxins.

In 2001, a University of California, Davis–University of the Republic, Montevideo, partnership created a new Fogarty ITREOH program to exploit the potential of ELISA to provide a low-cost approach to environmental analysis that could be attractive to the economically distressed countries of temperate South America. We describe the development and first results of the application of a locally developed ELISA method for microcystins, as well as our approach and progress thus far towards assisting local agencies to develop a regional network for monitoring Cyanobacteria blooms, especially in the Rio de la Plata, which forms the border between Argentina and southern Uruguay.

MATERIALS AND METHODS

Materials

Microcystins were obtained from Alexis Biochemicals (San Diego, CA) as MC-LR (purity ≥ 98.0 %); MC-RR (purity ≥ 97 %), and MC-LW (purity ≥ 95.0 %). The monoclonal antibody AD4G2 was kindly provided by Dr. Michael Weller from the Technical University of

ABBREVIATIONS

BSA	bovine serum albumin
DMSO	dimethylsulfoxide
FIC	Fogarty International Center
HRP	Horseradish peroxidase
IC ₅₀	midpoint of the standard curve of inhibition
MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry MC. microcystins
PBS	phosphate-buffered saline
PBS-T	PBS containing 0.05 % v/v Tween 20
TFA	trifluoroacetic acid

Munich (Germany). Goat anti-mouse IgG-horseradish peroxidase (HRP) conjugate was from Pierce Biotechnology (Rockford, IL), bovine serum albumin (BSA, fraction V, purity 98%) was obtained from Sigma-Aldrich (Saint Louis, MO). Purified water was prepared with a Milli-Q plus 185, (Millipore), C 18 cartridges, 500 mg C18-E (55 μ m, 70 A) were from Phenomenex, (Torrance, CA), and HPLC solvents were from J. T. Baker (Phillipsburg, NJ). All other chemicals were reagent or analytical grade.

Site Description

The coast of Montevideo, located in the southern cone of South America towards the northern side of the Rio de la Plata (Figure 1a), is characterized by an abundance of sand beaches. During the summer season, the water-quality monitoring program includes 20 bathing beaches, where water samples are obtained every other day and analyzed routinely for fecal coliforms. At the time of sampling the technician looks for algal blooms and, when algae are detected, takes samples for Cyanobacteria and toxin analysis. The background level is followed weekly by measuring chlorophyll *a*, even in the absence of detected algae. Samples were taken from 13 beach water sites of Montevideo; sites 7 and 11 are 200 m from the shoreline (Figure 1b), and site 14 is situated 200 km west of Montevideo.

Sampling, Phytoplankton and Chlorophyll *a* Analysis

Samples were collected in dark glass 1-L bottles and each was carefully subdivided into three aliquots for chlorophyll *a* determination, cell counting, and ELISA analysis. Aliquots for chlorophyll *a* determination and cell counting were stored refrigerated at 4° C until use (maximum preservation time eight hours). This study took place during the summers of 2004 and 2005: December 2003–March 2004 and December 2004–March 2005.

Cell counting was performed using Box's method,¹⁸ with disintegration of colonies by alkaline hydrolysis (80–90° C for 10 min, followed by intensive mixing). The isolated cells were counted using a counting chamber (Sedgewick-Rafter or a hemocytometer) with a 400 $\times$ microscope. In cases of low cell density it was necessary to concentrate with a 63- μ m (pore size) net for cell counting. The taxonomic observations were performed with a Leitz optic microscope. Phytoplankton analysis in water samples was done collaboratively with the National Direction of Aquatic Resources (DINARA, Montevideo, Uruguay) during the second summer season of this study (2004–2005).

Chlorophyll *a* was determined spectrophotometrically using cells filtered through glass fiber filters, then extracted in acetone:water 75:25, following APHA Method 10200h.¹⁹

Figure 1—*a)* Uruguay and South-America; *b)* Locations of sampling sites along the coast of Montevideo (sites 1–13). Site 14 is 200 km west of Montevideo.

Extraction and Cleanup

Water samples for the analysis of microcystins were stored at –20° C prior to use. To a 100-mL aliquot of this sample, 5% acetic acid (v/v) was added; after three cycles of freezing and thawing, the extract was centrifuged at 2,500 $\times$ g for 10 min. Unless otherwise specified, one aliquot of each sample (1 mL) was applied to the C18 cartridge preconditioned according to the manufacturer's specifications (10 mL methanol and 10 mL water). After sample application, the column was washed with 5 mL water followed by 5 mL of 10% (v/v) methanol, and the microcystin fraction was eluted with 2.5 mL of methanol. The microcystin fraction was preserved at –20° C and used as needed.

Indirect Competitive ELISA Assay

The ELISA assay was developed using the AD4G2 monoclonal antibody,²⁰ a kind gift of Dr. M. Weller (Munich, Germany). The coating antigen was prepared by coupling MC-LR to thiol groups introduced in cationized and thiolated bovine serum albumin.²¹ ELISA plates were coated with the MC-LR-microcystin–BSA conjugate (100 μ L per well containing 70 ng/mL of conjugate in PBS); then they were blocked with 200 μ L 1% BSA (m/v) in PBS, for 45 minutes and thoroughly washed with PBS-T. The mixture containing microcystin standards or adequately diluted samples in MilliQ water (50 μ L per well) and an optimized dilution of AD4G2 antibody supernatant (50 μ L per well) were incubated for one hour at 37° C. After further washing, the second antibody was added (100 μ L per well of anti-mouse-HRP conjugate diluted 1/5,000 in PBS-T) and the plate was incubated for one hour. The plates were washed and then 100 μ L of the peroxidase substrate (0.4 mL of 6 mg/mL DMSO solution of 3,3', 5,5'-tetramethylbenzidine, plus 0.1 mL of 1% H₂O₂ in water, prepared in a total volume of 25 mL of 0.1 M sodium acetate buffer pH 5.5) was dispensed into each well. The enzyme reaction was stopped after 30–40 min by the addition of 50 μ L of 2 N H₂SO₄. The absorbance was read at 450 nm in a Multiskan MS, Labsystems microtiter plate reader.

TABLE 2 Influence of Cleanup on the ELISA Result and Comparison of Local and Commercial ELISA*

		Local ELISA		Commercial ELISA	
Cleanup		($\mu\text{g/L}$)	SD ($\mu\text{g/L}$)	($\mu\text{g/L}$)	SD ($\mu\text{g/L}$)
Sample 1	No	260	35		
Sample 1	Yes	540	110	1,200	120
Sample 2	Yes	64	15	55	12
Sample 3	Yes	101	30	67	7

*The data are the averages of three independent determinations.

using a reference wavelength of 620 nm. Inhibition curves values were fitted to a four-parameter logistic equation using Microcal Origin version 6.0 (Microcal Software, Northampton, MA) package software.

HPLC Analysis

Extracts from three water samples (A, B, and C) were prepared and cleaned up using a preconditioned C18 cartridge as described. Proper dilutions of the extracts were applied to a Phenomenex Luna 5- μm C18 250 $\times$ 4.6-mm column using a Waters 1525 HPLC equipped with a 2487 UV-vis detector. The column was equilibrated with 70% H_2O , 0.05% TFA (buffer A) and 30% acetonitrile, 0.05% TFA (buffer B) and eluted with a two-step gradient. In the first step (15 min) the concentration of buffer B was raised to 50%, in the second step (6 min) the concentration of buffer B reached 90%. The outcome was followed by determining absorbance at 238 nm and the peaks corresponding to MC-RR, MC-LR, and MC-LW were identified using the appropriate standards. The main peaks were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).²²

RESULTS AND DISCUSSION

Rationale for Selection of ELISA

In Uruguay, the municipal laboratory in charge of monitoring recreational waters did not have the expertise and the equipment necessary to identify phytoplankton and to enumerate cyanobacterial cells unequivocally, so there was no capacity to characterize the risk of exposure to recreational waters in the presence of blooms. However, through an agreement with the University of the Republic–FIC Center, the lab was experienced in the use of non-commercial immunoassays.²³ Therefore, since it was known that the *Microcystis* was the predominant genus present,^{13,14} we attempted to develop and set up a simple ELISA assay to determine microcystins, which could be used to evaluate the possible risks associated with recreational use of local

beaches, by measuring the toxins. The main advantages of the ELISA assays are, in general, the ease of operation, low cost, high sensitivity, and in this case the broad specificity towards several of the main microcystin variants.²⁰ The use of commercial ELISA kits was not considered because of their prohibitively high cost (about US \$500 per ELISA plate).

ELISA Assay

In spite of a different format, the indirect ELISA parameters are similar to those reported for the original direct assay and showed an inter-assay average midpoint (IC_{50}) of $0.353 \pm 0.070 \mu\text{g/L}$ ($n = 5$, 1s), with a working range of 0.16–3.56 $\mu\text{g/L}$ (Figure 2). The antibody AD4G2 was raised against the Adda group; thus, its cross-reactivity with microcystins, nodularins, and peptide fragments containing Adda is very well characterized.²⁰

Toxin Extraction and Clean-Up

Toxins in water bodies during algal blooms are present both in the dissolved state (free, dissolved, or extracellular toxins) and inside the cyanobacterial cells (intracellular toxins). The consumption of inadequately treated drinking water, as well as the involuntary ingestion of raw water during bathing or swimming, exposes the target organism to the total toxin content. Thus, the analytical test must be able to determine both the intracellular and extracellular toxins (<http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/consultationconsult_intro_e.html>). Consequently, the extraction procedure must include the lysis or rupture of the algal cell wall to provide access for the extraction solvent to the intracellular toxin. Cell lysis can be performed a) by freeze-drying in combination with sonication in solution, b) by repeated freezing and thawing, or c) by sonication of whole cell material. The latter two methods were compared and the recoveries of microcystin after cell lysis of a model sample determined by ELISA were $41,200 \pm 6,300$ and $42,000 \pm 5,900 \mu\text{g/L}$, respectively, showing that the two procedures are equivalent, as determined in a sample obtained by concentration of a scum observed on December 2003. These values are the averages of two independent preparations for each procedure using triplicate analysis in high dilution (10^{-5}). Freezing and thawing was chosen for practical reasons in order to favor parallel processing of the samples. In many cases the high dilution used for the analysis of these extracts suppressed all matrix effects and allowed direct ELISA measurement of the microcystin content of filtered cells prepared from algal scums, without the need for sample cleanup (validated by extract spiking, data not shown). However, other samples, such as raw water and raw water with diluted cell suspensions, required the use of a clean-up step prior to analysis.

Figure 2—A standard curve for the non-commercial ELISA assay. Measurement of microcystin-LR with the monoclonal antibody AD4G2 in the indirect, competitive ELISA. Average parameters of five determinations are: slope 1.08 ± 0.21 AU/μg/L; midpoint (IC_{50}) 0.353 ± 0.07 μg/L; range of quantification, 0.16–3.56 μg/L.

The initial extraction can be done with water, organic solvents (such as methanol), or weak acid solutions (<http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/consultation/consult_intro_e.html>). The inclusion of methanol 75:25 in the extraction procedure did not show a significant difference as compared with the use of dilute acid alone. Thus, we selected the combination of freezing and thawing for cell rupture, with the use of dilute acetic acid for extraction, to keep the procedure as simple as possible. As mentioned before, matrix effects were observed for whole-water samples, e.g., samples containing cells and water with dissolved toxins. This is exemplified by the difference in toxin concentrations determined in sample 1 before and after the cleanup procedure (Table 2, local ELISA results). In these samples ELISA determination directly upon the samples after extraction was not possible even after 100–1,000-fold dilutions. Therefore, a sample cleanup procedure was added, which is also very convenient to avoid possible matrix effects due to the frequent changes in salinity and turbidity that occur during summers. The cleanup procedure by C18 solid-phase extraction cartridges showed a high recovery of spiked filtered samples (65–127%) and good repeatability (Table 2; Figure 3). Notice the overall similarity of the chromatograms in Figure 3, demonstrating the reproducibility of the cleanup procedure.

The Local ELISA

In two of the three samples studied, there was no significant difference between the commercial ELISA and the local ELISA assay (Table 2). In the case of sample 1, the local ELISA result was about 50% of that meas-

ured with the commercial kit. Since the recovery of microcystin in that spiked sample was good, we can point to differences in the cross-reactivity patterns of the antibodies against the different microcystin congeners present. Due to the fact that there are up to 90 MC variants and that the cross-reactivity for each variant can differ by factors of 2 and more, it is not to be expected that the results from two MC ELISA kits will necessarily agree for all samples. The point can be clarified by analyzing the congener compositions of samples from these blooms. The overall complexity of the samples is shown by the high-pressure liquid chromatography (HPLC) pattern and the main peaks have been studied by MS.

Detection by HPLC with UV is the most common method for quantification of microcystins. However, difficulties arise because excellent resolution of the different peaks is required and in addition, owing to the lack of standards, the identification of several peaks (which could represent different variants of microcystins) is problematic. Figure 4 shows the HPLC chromatograms of three representative extracts prepared from algal scums: samples A, B, and C are from the same blooms and sites as samples numbered 1 to 3, respectively, in Table 2. Under these conditions a good resolution of available standards (MC-LR, MC-RR, and MC-LW) was achieved, and a main peak corresponding to microcystin LR was detected in all samples. In addition, minor peaks at the retention time of the MC-RR variant, as well as several unidentified peaks, were obtained. No indication of MC-LW could be observed at this point. The numerous unidentified peaks could eventually account for different microcystin variants or for other compounds, but this cannot be clarified with UV-vis detection. For this reason, the main peaks were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).²¹ The presence of MC-LR, as well as that of MC-RR, could be confirmed in all samples, and no indication of MC-LW was found. MC-YR was present in all samples in the peak immediately before MC-LR, but that peak was not pure. The identity of the microcystins was further confirmed by post-source-decay (PSD) analysis.²² The PSD analysis showed that most of the unidentified peaks did not correspond to microcystins, and most peaks (except for the case of LR-MC) contained mixtures of compounds including several cyanopeptolins, which are not toxic. These other compounds may interfere with the quantification of microcystins by HPLC in routine analysis and highlight the relevance of ELISA based on AD4G2 antibody as an alternative/complementary, simple, and inexpensive method that gives specific information related to the presence of the AddA group, which can be more closely related to toxicity.

In the particular case of the samples used in this study, the local ELISA/HPLC comparison was rather good for the three samples tested, and these problems

Figure 3—Reproducibility of cleanup: HPLC analysis of two clean up replicates. The cleanup procedure was applied in parallel to two aliquots of the same algal scum sample, which were then run through a Phenomenex Luna 5- μ m C18 250 $\times$ 4.6-mm column as described in the text.

were not evidenced because of the large percentage of the MC-LR component present (Table 3). However, the different proportions of the other congeners in the samples may explain the differences in the results found with other ELISA kits.

Monitoring Microcystins

The content of microcystins was followed in the scums and in the bulk beach water. The Río de la Plata is a huge body of water that empties into the ocean waters, and the environmental conditions (currents, winds, etc.) are so dynamic that scum formation occurs suddenly and in most cases disappears after a few hours into the general flow. Since sampling and analysis take several hours, this means that by the time of reporting the toxin results, the situation may be very different at the beaches. Therefore, the concept was to analyze the possibility that warning to the public could be based on the use of visual detection parameters. Thus, it is necessary to collect enough data to analyze the correlation of these parameters with toxin concentration.

We have observed that disperse colonies of Cyanobacteria and of cyanobacterial scums are quite easy to identify by visual observation. Therefore, two broad categories were defined: a) “scum” and b) “disperse Cyanobacteria.” The scum can be seen from several meters distance, while the “disperse Cyanobacteria” can only be detected from a short distance (when

the observer is in the water that is being classified). Technicians and lifeguards were trained to distinguish between these two categories and representative samples of each condition were collected for analysis.

Summer of 2003–2004

During the first summer season only chlorophyll a and microcystins were determined in the affected areas, and the background levels between events were followed regularly (Table 4). The blooms usually appear at the end of the spring. The toxins reached maximum concentrations during the summer, and were mostly not detectable or present in low concentrations between the events. Usually, there are nondetectable levels during the winter and spring seasons. In the summer of 2004 (Dec. 2003–March 2004), the toxin concentration in the heavy scums was 63–3,800 μ g/g of dry scum (96–2,440 μ g/L water), and the ratio of microcystin/chlorophyll was in general higher than the average ratio of 0.4 reported for *Microcystis* blooms.²⁴ The salinity during summers is generally variable (5 to 25 salinity units) and the blooms usually appear after a drop in salinity, which reflects the fact that the cyanobacterial biomass is transported from the upper regions of the Río de la Plata where fresh water or lower salinities predominate. When the bloom occurred as disperse Cyanobacteria, microcystin concentrations ranged from nondetectable to 58 mg/L in that season.

Figure 4—HPLC analysis of three microcystin-containing extracts. Upper chromatogram displays the elution profile of the MC standards. A, scum sample from Montevideo, B and C, scum samples from site 14,200 km to the west of Montevideo. The putative peaks of MC-RR and MC-LR could be further confirmed by MALDI-TOF MS analysis.

Summer of 2004–2005

During the second summer studied, the number of cyanobacterial blooms, as well as the concentration of microcystins in scums, was notably lower than the values observed in 2003–2004 (Table 5). The highest values observed in scums (104 and 64 µg/L) were from a beach 200 km upstream of Montevideo, where salinity is much lower. When the blooms occurred as disperse *Cyanobacteria*, the maximum microcystin value observed was only 8.4 µg/L. That season was characterized by an important drought, and the average salinity (recorded daily) was higher than in the previous summer, which could explain the different behavior. Thus, the risk associated with recreational activities was lower than in the previous summer. In general, *Microcystis* species were present in the blooms, except for one case, in which *Microcystis* appeared together with *Anabaena* species.

Overall, the ratios microcystin/chlorophyll a, as well as the amounts of microcystin per cell, were quite vari-

able, which suggests that the measurement of chlorophyll a and/or cell enumeration alone is not a good indicator of the presence of microcystins. Therefore, to evaluate health risks associated with different uses of the affected water supplies, it is necessary to determine the toxin concentration. Concerning the microcystin results, in both seasons studied, 94.4% (17/18) of the samples classified as “disperse *Cyanobacteria*” presented values in the range of low or moderate probability of adverse effect on human health, according to WHO guidelines.²⁵ It is noteworthy that most samples classified as “scum” contained very high levels of toxin, representing a high probability of adverse effects on human health.

CONCLUSIONS AND FUTURE DIRECTIONS

With FIC ITREOH support, we have developed an ELISA test and sample preparation process for the analysis of microcystins in recreational waters of the

TABLE 3 Results with ELISA and HPLC Compared

	HPLC (µg/L)				ELISA (µg/L)	
	MC-RR	MC-LR	Σ MC	SD	Result	SD
Sample A*	190	815	1,010	44	1,350	90
Sample B	1.1	22.7	23.8	0.5	18	7
Sample C		123.0	123.0	3.3	111	10

*Samples A, B, and C are samples from the same blooms and sites as samples numbered 1 to 3, respectively, in Table 2.

TABLE 4 Summer 2003–2004: Microcystins and Chlorophyll in “Scums” and in Bulk Beach Water as “Disperse” Colonies of Cyanobacteria

Date	Microcystin Site	Microcystin (µg/L)	Chlorophyll (µg/g Dry)	(µg/L)	MC/Chlorophyll
Scum					
24/12/03	8	970	3,800	2,700	0.3
24/01/04	2	790			
27/01/04	9	96	63		
04/02/04	6	2,440	670		
04/02/04	1	780		1,700	0.5
Disperse					
10/01/2004	1	12		6	2.0
12/01/2004	12	9		41	0.2
27/01/2004	11	0.2		4	<0.1
04/02/2004	7	16		22	0.7
18/02/2004	2	32		11	2.9
10/03/2004	13	8		6	1.3
10/03/2004	10	< 0.16		4	<0.1
20/03/2004	4	58		51	1.1
21/03/2004	4	4		23	0.2
21/03/2004	3	2		18	0.1
21/03/2004	2	27		32	0.8
23/03/2004	3	< 0.16		2	<0.1
Reference value					0.4

Rio de la Plata. The test was validated by various techniques and was used to monitor the toxicity of Cyanobacteria blooms over two years. Our data indicate that the visual classification of blooms as “scums” or “disperse Cyanobacteria,” which can be established on site, appears to be a reasonably good practical criterion to make rapid decisions about the health risk of exposure to recreational waters accessible from the beaches of Montevideo. This criterion is being used by trained lifeguards, on each beach, to support recommendations to the public concerning the risks from bathing and swimming in the presence of scums. Considering that the blooms occurred about 20% of the days in both summer seasons, this represents an

important improvement in the capacity of the Municipal Laboratory of Montevideo to assist health authorities. Supplementary data collection over the coming seasons is necessary to more rigorously evaluate the validity of the proposed criteria.

Since cyanobacterial blooms are a common problem in the region, we are joining efforts with a local NGO called “FREPLATA” (Protección Ambiental del Río de la Plata y su Frente Marítimo), a cooperative initiative of Uruguay and Argentina (<<http://www.freplata.org>>) to disseminate the use of the assay. Several workshops, short courses, and symposia on Cyanobacteria, as well as ELISA training courses, have been organized. A three-day course was presented in Montevideo Novem-

TABLE 5 Summer 2004–2005: Microcystins, Chlorophyll a, Cell Enumeration in “Scums” and Bulk Beach Water in “Disperse” Colonies of Cyanobacteria.

Date	Site	MC µg/L ⁻¹	Chlorophyll (µg/L)	Cells (10 ⁻³ /mLL)	MC/Cell pg/C	MC/Chlorophyll	Chlorophyll pg/cell
Scum							
27/12/2004	10	22	2,420	743	<0.1	<0.1	3.3
24/01/2005	14	64	230	630	0.1	0.3	0.4
19/11/2004	12	40	310	5,300	<0.1	0.1	<0.1
18/11/2004	12	39	56	450	<0.1	0.7	0.1
15/02/2005	14	104	890	2,460	<0.1	0.1	0.4
Disperse							
14/12/2004	2	0.4	33	73	<0.1	<0.1	0.5
14/12/2004	3	2.0	15			0.1	
14/12/2004	10	< 0.16	4			<0.1	
26/02/2005	13	8.4	134	71	0.1	<0.1	1.9
26/02/2005	5	5.7	9			0.6	
26/02/2005	6	< 0.16	9			<0.1	
Reference value					0.2	0.4	0.5

ber 21–23, 2005, with 18 full participants and an international faculty from Uruguay, Brazil, and Argentina (<<http://mail.fq.edu.uy/~inmuno/curso%20microcistinas/comenzar%20aca.htm>>). Three of the authors (NF, LD, DS) were FIC trainees.

Regarding the transfer of the ELISA test, it has been adapted into a kit format with pre-coated plates to facilitate its transport to regional control centers, and it will be further tested and validated in waters of the Argentinean coast of the Rio de la Plata. The cost of the local ELISA is less than 5% that of the commercial kit, and the stock available, as well as the stability of reagents (preserved at -20°C), will allow its use in this region for several years. With additional FIC ITREOH support and training, we have also developed the capacity for large-scale development and manufacture of local ELISA kits at the University of the Republic's Faculty of Chemistry, so that programs such as this one can be continued in the future.

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