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Early warning genetic testing of toxic cyanobacteria in water supply

Abstract

This invention is at least one panel of qPCR/RT-qPCR assays which enables simultaneous testing for the presence of multiple species and subgroups of cyanobacteria that produce microcystin, anatoxin, saxitoxin, and cylindrospermopsin cyanotoxins. The method takes into account that some cyanobacteria species may carry genes associated with multiple toxin types. Testing for each toxin type is conducted under standardized test conditions which allow quantification of the number of gene copies present for cyanotoxins which may contribute to the overall toxin level.

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Background/Summary

INCORPORATION OF SEQUENCE LISTING

(1) Incorporated by reference in its entirety herein is a computer-readable nucleotide sequence listing submitted concurrently herewith and identified as follows: One 12,418 Byte ASCII (Text) file named “EPA_800-17_SEQ.txt,” created on Aug. 29, 2018.

FIELD OF INVENTION

(2) The present invention relates to nucleic acid products used in the analysis of nucleic acids, such as primers or probes for detection or identification of organisms for bacteria, and more specifically to an assay for simultaneously conducting testing for a plurality of cyanobacteria which carry a gene to produce a toxin, using standardized test conditions.

BACKGROUND OF THE INVENTION

(3) The U.S. Environmental Protection Agency (EPA) publishes an annual list of the top thirty unregulated contaminants that are known or expected to occur in public water systems in the U.S. Ten of the thirty contaminants of concern are toxins produced by a common type of bacteria called cyanobacteria.

(4) Cyanobacteria, also called blue-green algae, are microscopic organisms found naturally in all types of water. A “cyanobacterial bloom” is an event during which cyanobacteria, multiply very quickly. Blooms can form in warm, slow-moving waters that are rich in nutrients from fertilizer runoff or septic tank overflows, and most often occur in late summer or early fall.

(5) A harmful cyanobacterial bloom is an event associated with elevated cyanotoxin levels that are either deemed unsafe or require further monitoring. Exposure to high cyanotoxin levels causes damage to liver cells and neural signaling pathways in humans, as well as less severe effects such as skin rashes. These events occur on a global basis at great risk to local populations. For example, in 2014, a toxic bloom left 500,000 people in Ohio without drinking water. In 2014, a toxic bloom caused Florida to declare a state of emergency.

(6) Most cyanobacterial blooms do not produce toxins at a sufficient level to compromise public water supplies and cause harm to humans and other species. Additionally, the vast majority of cyanobacteria species do not carry the gene necessary to produce toxins. However, several types of cyanobacteria carry genes which produce one or more types of toxins during a bloom. The aggregate level of all types of cyanotoxins produced by all species known to be carriers may cause the toxin level to exceed a safe threshold for humans and other species.

(7) Historically, water supplies have been monitored by measuring cyanobacteria count and biomass to determine the presence of cyanobacterial species and their blooms, without differentiating species that carry harmful toxin genes or the types of toxins produced.
(8) More recently, assays have been developed to test for genes associated with microcystin, anatoxin, saxitoxin and cylindrospermopsin by performing quantitative polymerase chain reaction (qPCR) and reverse transcription qPCR (RT-qPCR) methods known in the art. These test methods known in the art can detect the presence of a single toxin gene type produced by multiple species.
(9) RT-qPCR and qPCR testing must be performed under different time and temperature conditions for each toxin type, and testing methods must be further differentiated for individual species. The number of gene copies detected can be correlated to future levels for the individual toxin. However, the test conditions of each assay used to test an individual toxin gene are not uniform, and the quantifications produced using each assay are not statistically comparable. Therefore, the results cannot be aggregated to predict a total toxin level.
(10) There is an unmet need for a single assay kit which can simultaneously test for multiple common types of cyanotoxin producing genes across diverse species. There is a further unmet need for testing methods that can be used to accurately determine the probability that a cyanobacteria bloom will result in cyanotoxin levels that exceed US EPA 10-day health advisory levels for drinking water.

SUMMARY OF THE INVENTION

(11) This invention is a system comprised of quantitative polymerase chain reaction (qPCR) and reverse transcription qPCR (RT-qPCR) assays for detecting the presence of cyanotoxin genes. In various embodiments, the invention includes one or two panels of assays for screening and early warning and for toxic group identification.
(12) The invention enables simultaneous testing for the presence of cyanobacteria genes associated with four toxins which contribute to the cyanotoxin level in a water supply, and the presence of dominant toxic cyanobacterial groups which may trigger the need for an EPA Health Advisory alert. The toxin types associated with the tested genes are anatoxin, microcystin, saxitoxin and cylindrospermopsin. The invention includes qPCR assay panels comprised of separate assays to simultaneously detect each toxin type and toxic group. The assays operate under standardized test conditions. The standardized test conditions include a uniform annealing temperature, thermocycle duration and control samples having common parameters. Results obtained using these standardized conditions can be used to determine the aggregate number of gene copies present in a sample for the four toxin types tested.
(13) In various embodiments, the assay contained in each assay panel has a common annealing temperature of approximately 62 degrees Celsius.
(14) In various embodiments, the method of use includes steps for producing mathematically comparable test results for each toxin quantifying the number of cyanotoxin gene copies detected by each assay and producing test data which may be aggregated for multiple toxin types.

TERMS OF ART

(15) As used herein, the term, “aggregate number of gene copies” means the total number of gene copies present in a sample for the four toxins tested which contribute to overall toxin levels.
(16) As used herein, the term, “comparable test results” means test data which is obtained under standardized test conditions so that it is mathematically comparable and may be aggregated and analyzed relative to multiple toxin types.
(17) As used herein, the term, “standardized test conditions” means a set of common parameters for multiple qPCR/RT-qPCR tests which results in comparable test results.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

(1) FIG. **1** is a diagram which illustrates how a Cyanotoxin Prediction (CTP) Assay Panel can be used to more accurately detect the presence of toxin-producing cyanobacteria in a water sample.

(2) FIG. **2** is a table identifying multiple cyanotoxin types, the carrier group of cyanobacteria associated with each cyanotoxin gene, and the common DNA sequences which define a member of the cyanobacteria carrier group.

(3) FIG. **3** is a table illustrating exemplary primer and probe sequences which can be used to produce a CTP Assay Panel for early detection and warning.

(4) FIG. **4** is a table illustrating primer and probe sequences which can be used to produce a CTP Assay Panel for detecting dominant toxic groups of cyanobacteria.

(5) FIG. **5** illustrates the common genetic sequences which are detected by the primers and probes used to produce a CTP Assay Panel for detecting dominant toxic groups of cyanobacteria.

(6) FIG. **6** illustrates an exemplary method of using a CTP Assay Panel and correlating CTP Assay Panel results to subsequently measured cyanotoxin levels.

(7) FIG. **7** summarizes exemplary data reporting the number of toxic gene copies and the concentration of cyanotoxins measured during periodic testing of a water source.

(8) FIG. **8** summarizes exemplary data correlating the number of toxic gene copies measured by the CTP Assay Panel to the subsequent concentration of cyanotoxins measured by an enzyme-linked immunosorbent assay (ELISA).

DETAILED DESCRIPTION OF THE INVENTION

(9) FIG. **1** is a diagram which illustrates how one exemplary embodiment of a Cyanotoxin Prediction (CTP) Assay Panel can be used to more accurately detect the presence of toxin-producing cyanobacteria in a water sample.

(10) FIG. **1** illustrates four known types of cyanotoxins (microcystin, anatoxin, saxitoxin, and cylindrospermopsin) which are produced by more than one subgroup of cyanobacteria, represented as ovals. Each subgroup of cyanobacteria includes multiple species, represented as circles; however, only a few of these species produce cyanotoxins. Species carrying toxic genes that produce cyanotoxins are represented by an asterisk or symbol in the circle.

(11) In the exemplary embodiment shown, the CTP Assay Panel distinguishes between toxic and non-toxic species to specifically detect the presence of toxic species.

(12) The CTP Assay Panel identifies and distinguishes the presence of toxic subgroups of cyanobacteria through the use of novel oligonucleotide primers and quantitative polymerase chain reaction (qPCR) amplification methods known in the art.

(13) FIG. **2** is a table identifying multiple cyanotoxins, the carrier group of cyanobacteria associated with each cyanotoxin gene, and the common DNA sequences which define a member of the cyanobacteria carrier group.

(14) The right-most column illustrates the common DNA sequences identified by the invention. These sequences, also called consensus sequences, are common in multiple species and allow simultaneous testing for four different toxin genes to simultaneously detect the presence of multiple species that produce cyanotoxins.

(15) FIG. **3** is a table illustrating exemplary primer and probe sequences which can be used to produce CTP Assay Panel 100 for early detection and warning.

(16) In one exemplary embodiment, CTP Assay Panel 100 is a panel of RT-qPCR/qPCR assays for detecting cyanotoxin genes, which include the novel primer pairs described in FIG. **3**. In this embodiment, the primer pairs are designed to detect multiple species of toxic cyanobacteria simultaneously. The primers shown each have a sequence that will bind to a cyanotoxin gene at 60-64 degrees Celsius. In various embodiments, the recommended annealing temperature for the primers shown is 62 degrees Celsius. This common annealing temperature allows all assays to be conducted simultaneously. Without these novel primers and standardized conditions for multiple

species, it was not possible to integrate all of the test results for multiple species. Standardized qPCR reaction conditions produce statistically comparable qPCR data from samples with different species, taken from geographically diverse waters.

(17) In this exemplary embodiment, the assays are standardized with the same common annealing temperature, thermocycle duration, and control samples designed to yield consistent qPCR test results. In various embodiments, CTP Assay Panel 100 further includes approximately four to six positive control samples, each having a unique number of cyanotoxin gene copies within a range of approximately 1,000 to 10,000 DNA gene copies per liter.

(18) In one embodiment, simultaneous detection of the mcyE/mcyA, sxtA, cyrA, or anaC genes indicates possible production of microcystin, saxitoxin, cylindrospermopsin or anatoxin, respectively. In this exemplary embodiment, the RT-qPCR/qPCR assay detects the presence of cyanotoxin genes in control samples and collected water samples or other test samples. In various embodiments, CTP Assay 100 can be used to determine the total number of gene copies for each cyanotoxin gene and estimate the population size of each group of toxic cyanobacteria.

(19) In the exemplary embodiment shown, each primer pair selected for qPCR analysis targets a sequence of cyanotoxin biosynthesis genes and genus-specific genes that is common to multiple cyanobacteria species. The target genes encode cyanotoxins, including microcystin, anatoxin, saxitoxin, and cylindrospermopsin. Targeted genes include an mcyA gene sequence carried by cyanobacteria in all six genera, an anaC gene sequence carried by cyanobacteria in the *Anabaena* and *Aphanizomenon* genera (exemplary detected species include *Aphanizomenon gracile, Anabaena* sp., and *Anabaena circinalis*), an sxtA gene sequence carried by cyanobacteria in the *Anabaena*and *Aphanizomenon* genera, and a cyrA gene sequence carried by cyanobacteria in the *Anabaena, Aphanizomenon, Cylindrospermopsis*, and *Raphidiopsis* genera (exemplary detected species include *Raphidiopsis curvata* and *Cylindrospermopsis raciborskii*).

(20) FIG. **4** is a table illustrating primer and probe sequences which can be used to produce CTP Assay Panel 200 for identifying dominant toxic groups of cyanobacteria.

(21) CTP Assay Panel 200 can detect multiple toxic species simultaneously. In various embodiments, CTP Assay Panel 200 can detect the number of toxic gene copies and predict the level of toxin that will be produced by each type of cyanobacteria individually and in the aggregate.

(22) In an alternative embodiment, CTP Assay Panel 200 is comprised of a panel of multiple RT-qPCR/qPCR assays that include the primers shown in FIG. **4**A. In this embodiment, the RT-qPCR/qPCR assay detects the presence or absence of individual toxin-producing subtypes of cyanobacteria to determine dominant toxic groups in control samples and collected water samples or other test samples. This embodiment more specifically determines which individual subtypes of cyanobacteria are present and which has the highest population. Each primer shown has an annealing temperature of approximately 60 to 64 degrees Celsius. In various embodiments, the recommended annealing temperature for the primers shown is 62 degrees Celsius.

(23) In the alternative embodiment, alternative primer pairs can detect an mcyA or mcyE gene sequence carried by cyanobacteria in the *Anabaena, Nostoc, Microcystis, Planktothrix*, and *Synecococcus* genera (exemplary detected species include *Anabaena* sp., *Anabaenopsis elenkinii, Anabaena lemmermannii, Anabaena flos-aquae, Nostoc* sp., *Fischerella* sp., *Nodularia spumigena, Nodularia sphaerocarpa, Nodularia* sp., *Microcystis* sp., *M. aeruginosa, M. viridis, M. panniformis, M. wesenbergii, M. smithii, Planktothrix* sp., *P. rubescens, P. agardhii, Synechococcus* sp., WH 8103, and WH8102), an anaC gene sequence carried by cyanobacteria in the *Anabaena*, and *Aphanizomenon* genera, an sxtA gene sequence carried by cyanobacteria in the *Aphanizomenon* genus, a cyrA gene sequence carried by cyanobacteria in the *Anabaena, Aphanizomenon, Cylindrospermopsis*, and *Raphidiopsis* genera (exemplary detected species include *Raphidiopsis curvata* and *Cylindrospermopsis raciborskii*), a geoA gene sequence carried by cyanobacteria in the *Anabaena* and *Aphanizomenon* genera (exemplary detected species include *Dolichospermum ucrainicum, D. planctonicum, D. circinale, Nicotiana attenuate*, and *Anabaena ucrainica*), a pstS

phosphase gene sequence carried by cyanobacteria in the *Anabaena* and *Aphanizomenon* genera, and a nif gene sequence carried by cyanobacteria in the *Anabaena* and *Nostoc* genera.
(24) FIG. **5** illustrates the common genetic sequences which are detected by the primers and probes used to produce CTP Assay Panel 200 for identifying dominant toxic groups of cyanobacteria.
(25) FIG. **5** illustrates the common DNA sequences which define a member of the cyanobacteria carrier group detected by the CTP Assay Panel 200 primers and probes.
(26) The exemplary common microcystin sequences shown in FIG. **2** and FIG. **5** are listed as <SEQ ID NO. 52> through <SEQ ID NO. 61> in the sequence listing file. Exemplary common anatoxin sequences are listed as <SEQ ID NO. 64>. Exemplary common saxitoxin sequences are listed as <SEQ ID NO. 65> and <SEQ ID NO. 66>. Exemplary common cylindrospermopsin sequences are listed as <SEQ ID NO. 67>. Exemplary common geoA sequences are listed as <SEQ ID NO. 62> and <SEQ ID NO. 63>.
(27) FIG. **6** illustrates exemplary Method **300** for using CTP Assay Panel 100 and/or 200 to measure cyanotoxin genes and correlating CTP Assay results to subsequently measured cyanotoxin levels.
(28) In the exemplary embodiment shown, Method **300** utilizes a panel of novel qPCR/RT-qPCR assays for simultaneously detecting microcystin, anatoxin, saxitoxin, and cylindrospermopsin genes in cyanobacteria. The invention is a testing method for detecting specific bacterial groups associated with toxin production.
(29) In various embodiments, Method **300** may be used to identify the number of gene copies present and predict the amount of toxin that will be produced by each cyanobacteria genus individually and in the aggregate. In various embodiments, Method **300** utilizes analysis of the qPCR/RT-qPCR results to predict whether cyanotoxin concentrations in a source of water will be exceed a toxic threshold deemed harmful to humans and other species within a specified period of time. In various embodiments, the toxic threshold is a limit set by U.S. EPA Drinking Water Health Advisories. For example, the threshold for combined microcystin toxins is 0.3 μg/liter and a gene copy number of 1,000 to 10,000 DNA gene copies per liter predicts that the toxic threshold will be exceeded seven days after measuring the gene copy number.
(30) Step **1** is the step of collecting water samples. In various embodiments, this step is accomplished by periodically collecting water samples from the same source, at various points in time.
(31) Step **2** is the step of isolating genetic material from a water sample.
(32) In one exemplary embodiment this step is accomplished by dividing samples 100-300 mL aliquots and individually filtering the aliquots using EMD Millipore DuraporeTM membrane filters (0.40 μm, MilliPore, Foster City, CA) for DNA extraction. In one embodiment, DNA and RNA are extracted using a kit known in the art, such as AllPrep DNA (QIAGEN, Valencia, CA). Filtered aliquots are stored at −80° C. in 1.5 mL microtubes with lysis buffer prior to extracting DNA and RNA.
(33) In various embodiments, this step includes using any method known in the art for isolating or extracting genetic material from a water sample and conducting reverse transcription to create template DNA from RNA.
(34) Step **3** is the step of using CTP Assay Panel 100 and/or 200 to determine the number of copies of toxic genes.
(35) To conduct a qPCR/RT-qPCR assay, components are combined and heated to create a polymerase chain reaction. In one exemplary embodiment, each reaction contains 1 μM concentration of each selected primer, 2 μl of template DNA from either the sample or the control, a 0.2 mM concentration of each of the four deoxynucleoside triphosphates (dTTP, dCTP, dGTP, and dATP), 1.5 mM MgCl2, 1 μM (each) primer, and 2.5 U of TaqDNA polymerase (Clone Tech, Mountain View, CA) in a total volume of 25 μl. In various embodiments, the effective primer concentration range for the PCR reaction is approximately 0.5 to 1 μM. In this embodiment, the

reactions are heated and cooled during 25 cycles of temperature changes, wherein each cycle includes 1 minute of denaturation at 94° C., 1 minute of primer annealing at 62° C., and 5 minutes of primer extension at 72° C. In various embodiments, the annealing temperature is approximately 60 to 64° C.

(36) In various embodiments, this step further includes analyzing the results by methods known in the art to determine the gene copy number (X.sub.gene) in each sample, for each cyanotoxin gene detected in that sample. In various embodiments, this step may include running CTP Assay Panel 100 and/or 200 on a Juno robot platform where 40 assays can be run at one time, including 1,600 reactions.

(37) Step **4** is the optional step of validating CTP Assay Panel 100 and/or 200 results by measuring toxin concentration levels on a subsequent date using a testing method known in the art and comparing the measured toxin concentration levels to the results of CTP Assay Panel 100.

(38) FIG. **7** summarizes exemplary data reporting the number of toxic gene copies and the concentration of cyanotoxins measured during periodic testing of a water source. FIG. **7** summarizes the number of toxic gene copies in DNA isolated from a water source and measured once per week between May 6 and September 30. Data marked LogMS2R were measured by a quantitative polymerase chain reaction (qPCR) assay (represented by squares) and data marked LogMS2Rrt were measured by a reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay (represented by circles).

(39) In the exemplary embodiment shown, the concentration of cyanotoxins in a water source was measured by an enzyme-linked immunosorbent assay (ELISA), represented by diamonds. The raw concentration of cyanotoxins measured by ELISA is represented by triangles.

(40) The x-axis shows dates and the y-axis shows gene copy number or toxin concentration on a logarithmic scale.

(41) In alternative embodiments, the concentration of cyanotoxins in a water source is measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

(42) FIG. **8** summarizes exemplary data correlating the number of toxic gene copies measured by CTP Assay Panel 100 and/or 200 to the subsequent concentration of cyanotoxins measured by an enzyme-linked immunosorbent assay (ELISA).

(43) FIG. **8** shows a regression, which is the best fit curve, of the correlation between the number of toxic gene copies measured by qPCR and RT-qPCR and the concentration of cyanotoxins in a water source measured by an enzyme-linked immunosorbent assay (ELISA). FIG. **8** demonstrates that there is a direct correlation between the expression of the toxic gene and the production of the toxin. The gene copy number is plotted on the x-axis on a logarithmic scale and the associated cyanotoxin concentration measured seven days after the gene copy number from the same water source is plotted on the y-axis on a logarithmic scale. In the exemplary embodiment shown, measured data are represented by open circles. The best fit curve showing a predicted toxin concentration on the y-axis for a given gene copy number measured by CTP Assay Panel 100 or 200 is represented by a dark, thin solid line. The 95% confidence interval of the best fit curve is represented by a thicker, lighter line. The 95% prediction limits showing a range of toxin concentration levels predicted by a given gene copy number are represented as dashed lines.

Claims

1. A qPCR and/or RT-qPCR cyanotoxin detection apparatus, comprising: a first set of primers, comprising a first primer consisting of the sequence set forth in SEQ ID NO:1 and a second primer consisting of the sequence set forth in SEQ ID NO:2; a second set of primers, comprising a third primer consisting of the sequence set forth in SEQ ID NO:21 and fourth primer consisting of the sequence set forth in SEQ ID NO:22; a third set of primers, comprising a fifth primer consisting of the sequence set forth in SEQ ID NO: 23 and a sixth primer consisting of the sequence set forth in

SEQ ID NO:24; a fourth set of primers, comprising a seventh primer consisting of the sequence set forth in SEQ ID NO:27 and an eighth primer consisting of the sequence set forth in SEQID NO: 28, and at least one probe comprising a detectable label.

2. The apparatus of claim 1, wherein said sets of primers are each present in the apparatus at a concentration of 1 μM.

3. The apparatus of claim 1, wherein said primers hybridize under conditions in which oligonucleotide hybridization occurs with cyanobacterial genes produced by bacteria selected from the group consisting of: *Microcystis, Nostoc, Planktothrix, Synecococcus, Anabaena, Aphanizomenon, Raphidiopsis, Cylindrospermopsis, Anabaena* sp., *Anabaenopsis elenkinii, Anabaena lemmermannii, Anabaena flos-aquae, Nostoc* sp., *Fischerella* sp., *Nodularia spumigena, Nodularia sphaerocarpa, Nodularia* sp., *Microcystis* sp., *M. aeruginosa, M. viridis, M. panniformis, M. wesenbergii, M. smithil, Planktothrix* sp., *P. rubescens, P. agardhii, Synechococcus* sp., *Raphidiopsis curvata, Cylindrospermopsis raciborskil, Dolichospermum ucrainicum, D. planctonicum, D. circinale, Nicotiana attenuate, Anabaena ucrainica, Aphanizomenon gracile*, and *Anabaena circinalis.*

4. A method of detecting the presence of toxic cyanobacterial genes in fresh water supplies, which comprises: providing a qPCR and/or RT-qPCR cyanotoxin detection apparatus, wherein said cyanotoxin detection apparatus comprises: a first set of primers, comprising a first primer consisting of the sequence set forth in SEQ ID NO:1 and a second primer consisting of the sequence set forth in SEQ ID NO:2; a second set of primers, comprising a third primer consisting of the sequence set forth in SEQ ID NO:21 and fourth primer consisting of the sequence set forth in SEQ ID NO:22; a third set of primers, comprising a fifth primer consisting of the sequence set forth in SEQ ID NO:23 and a sixth primer consisting of the sequence set forth in SEQ ID NO:24: a fourth set of primers, comprising a seventh primer consisting of the sequence set forth in SEQ ID NO:27 and an eighth primer consisting of the sequence set forth in SEQ ID NO:28, and at least one probe comprising a detectable label; and performing the steps of simultaneously: performing a first qPCR assay with the first set of primers for detecting a microcystin gene; performing a second qPCR assay with the second set of primers for detecting an anatoxin gene; performing a third qPCR assay with the third set of primers for detecting a saxitoxin gene; and performing a fourth qPCR assay with the fourth set of primers for detecting a cylindrospermopsin gene.

5. The method of claim 4, which further comprises the step of selecting at least one primer pair for detecting a microcystin gene from the group of primer pairs consisting of: forward primer consisting of the sequence set forth in SEQ ID NO:1 and reverse primer consisting of the sequence set forth in SEQ ID NO:2; forward primer consisting of the sequence set forth in SEQ ID NO:3 and reverse primer consisting of the sequence set forth in SEQ ID NO:4; forward primer consisting of the sequence set forth in SEQ ID NO:5 and reverse primer consisting of the sequence set forth in SEQ ID NO:6; forward primer consisting of the sequence set forth in SEQ ID NO:7 and reverse primer consisting of the sequence set forth in SEQ ID NO:8; forward primer consisting of the sequence set forth in SEQ ID NO:9 and reverse primer consisting of the sequence set forth in SEQ ID NO10; forward primer consisting of the sequence set forth in SEQ ID NO:11 and reverse primer consisting of the sequence set forth in SEQ ID NO:12; forward primer consisting of the sequence set forth in SEQ ID NO:13 and reverse primer consisting of the sequence set forth in SEQ ID NO:14; forward primer consisting of the sequence set forth in SEQ ID NO:15 and reverse primer consisting of the sequence set forth in SEQ ID NO:16; forward primer consisting of the sequence set forth in SEQ ID NO:17 and reverse primer consisting of the sequence set forth in SEQ ID NO:18; and forward primer consisting of the sequence set forth in SEQ ID NO:19 and reverse primer consisting of the sequence set forth in SEQ ID NO:20.

6. The method of claim 4, which each primer is present at concentration of 1 μM.

7. The method of claim 4, which further comprises obtaining one or more values reflecting a copy number, X.sub.gene, of at least one cyanotoxin gene present in at least one of said fresh water

supplies, wherein said cyanotoxin gene is selected from a group consisting of microcystin, anatoxin, saxitoxin, and cylindrospermopsin.

8. The apparatus of claim 1, wherein the apparatus further comprises a membrane filter and a DNA extraction kit, wherein the membrane filter possesses a pore size of 0.40 μm.

9. The apparatus of claim 2, wherein the apparatus further comprises: (i) dTTP, dCTP, dGTP, and dATP, (ii) MgCl.sub.2, and (iii) TaqDNA polymerase.

10. The apparatus of claim 8, wherein the apparatus further comprises: (i) dTTP, dCTP, dGTP, and dATP, (ii) MgCl.sub.2, and (iii) TaqDNA polymerase.

11. A kit for detecting the presence of cyanobacterial toxins in a sample by qPCR and/or RT-qPCR assay, comprising: a first set of primers, comprising a first primer consisting of the sequence set forth in SEQ ID NO: 1 and a second primer consisting of the sequence set forth in SEQ ID NO:2; a second set of primers, comprising a third primer consisting of the sequence set forth in SEQ ID NO:21 and fourth primer consisting of the sequence set forth in SEQ ID NO:22; a third set of primers, comprising a fifth primer consisting of the sequence set forth in SEQ ID NO: 23 and a sixth primer consisting of the sequence set forth in SEQ ID NO:24; a fourth set of primers, comprising a seventh primer consisting of the sequence set forth in SEQ ID NO:27 and an eighth primer consisting of the sequence set forth in SEQIDNO: 28; a mixture of dTTP, dCTP, dGTP, and dATP, a solution of MgCl.sub.2, TaqDNA polymerase, and at least one probe comprising a detectable label, wherein the cyanobacterial toxins are microcystin, anatoxin, saxitoxin, and cylindrospermopsin.

12. The kit of claim 10, wherein the primers in each of the sets of primer are present in an amount of 1 μM each.

13. The kit of claim 11, further comprising a membrane filter and a DNA extraction kit, wherein the membrane filter possesses a pore size of 0.40 μm.

14. The kit of claim 11, further comprising one or more positive control samples corresponding to each of the cyanobacterial toxins.

15. The kit of claim 11, further comprising four to six positive control samples corresponding to each of the cyanobacterial toxins.

16. The kit of claim 11, wherein the kit detects the presence of the cyanobacterial toxins in a single reaction under a single set of reaction conditions comprising an annealing temperature and thermocycle duration.

17. The kit of claim 15, wherein the presence of cyanobacterial toxins is detected by quantitation of an aggregate number of gene copies corresponding to the cyanobacterial toxins present in the sample.