Guide for Analyzing UCMR4 Samples in Compliance with EPA Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA Enzyme-Linked Immunosorbent Assay

SECTION 1: INTRODUCTION

In October of 2016, the US EPA released *Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA Enzyme-Linked Immunosorbent Assay* (ELISA). What follows is Gold Standard Diagnostics Horsham's interpretation of the method with instructions for the collection/preservation of samples and preparation of the expanded quality control samples required by the method. Guidelines for how to perform the Initial Demonstration of Capability, which each analyst must successfully perform before becoming certified to analyze samples in accordance with the method, are also included. The IDC contains four sections: demonstration of precision and accuracy, demonstration of acceptable system background, confirmation of the Minimum Reporting Level (for which the EPA has set a concentration of 0.30 ppb), and confirmation of a quality control sample.

Although the following procedures use Gold Standard Diagnostics Horsham's ABRAXIS® products that the client may be familiar with already, it is important that laboratory analysts, or anyone required to perform this method, read the procedures very carefully to ensure full understanding and compliance with the requirements of Method 546.

SECTION 2: GLOSSARY OF TERMS

Acceptable Sample Background (ASB) LRB control – A control that is analyzed during the IDC to demonstrate that the sample background and extraction processes are not contributing bias to the results. This control is frozen, thawed, and filtered along with the other samples and controls.

Analysis Batch – A batch of standards, samples, and all required controls on a single 96 well plate.

Initial Demonstration of Capability (IDC) – An Analysis Batch with additional controls that are used to prove an analyst can accurately analyze samples in accordance with Method 546. This must be performed successfully before an analyst is certified to analyze samples per Method 546.

IDC MRL Sample – A sample that is prepared at the MRL concentration during the IDC. The sample must be lysed and filtered.

Laboratory Fortified Blank (LFB) – An aliquot of reagent water to which a known concentration of MC-LR is added. This is a control that appears on each Analysis Batch to verify method performance in the absence of sample matrix. Two LFBs must be included in every Analysis Batch. LFBs have a concentration of 0.6 ppb and an acceptable range of ≥ 0.36 ppb and ≤ 0.84 ppb. The LFB is prepared by the testing facility using the ABRAXIS® 50 ppb MC-LR Spiking Solution contained in the ABRAXIS® Method 546 Accessory Pack.

Laboratory Fortified Sample Matrix (LFSM) and Laboratory Fortified Sample Matrix Duplicate (LFSMD) – Identical aliquots of a new or previously analyzed field sample to which a known concentration of MC-LR is added. This is a control that must be included in each Analysis Batch and is used to determine if the sample matrix contributes bias to the results. An LFSM must be analyzed with an accompanying LFSMD (see below). A representative LFSM and LFSMD set must be analyzed for each type of sample (ambient (source) water or drinking (finished) water) in the analysis batch. If both ambient and drinking water samples are present in a sample Analysis Batch, at least one LFSM/LFSMD

set must be present for each sample type. If analyzing more than 20 samples of either type, a second LFSM/LFSMD set must be analyzed for that type of sample. The LFSM is used to verify precision in both ambient and drinking water matrices.

Laboratory Reagent Blank (LRB) – An aliquot of reagent water that is lysed and filtered according to the same procedure used for sample processing. It is used to determine if interferences are introduced during the analytical process.

Low Range Calibration Verification (Low-CV) – A control with a concentration that is less than or equal to the MRL. The Low-CV is not lysed or filtered before analysis. It is used to confirm the accuracy of concentrations near the MRL. Low-CVs are fortified at 0.3 ppb and have an acceptable recovery range of ≥ 0.15 and ≤ 0.45 ppb. A Low-CV control is included in the ABRAXIS® Method 546 Accessory Pack, but it may also be prepared by the analyst.

Method 546 Quality Control Sample (546 QCS) – A control that is prepared using source material that is different from the calibration standard (second source). This is used to determine the accuracy of the calibrators. It is analyzed with each new lot of calibration standards and with each Initial Demonstration of Capability. The Method 546 QCS has a concentration of 0.6 ppb and an acceptable range of ≥ 0.42 ppb and ≤ 0.78 ppb. A Method 546 QCS is included in the ABRAXIS® Method 546 Accessory Pack. **Note: The Method 546 QCS is not the same concentration as the QCS contained in the ABRAXIS® Microcystins/Nodularins-ADDA kits, PNs 520011, 520011OH, and 520011SAES.**

Minimum Reporting Level (MRL) – The minimum concentration that can be quantified accurately and can therefore be reported. The EPA has set the concentration at 0.30 ppb.

Precision and Accuracy Standards (P&A) – Standards that are analyzed during the IDC to determine if an analyst can meet the requirements set forth by Method 546. P&A standards are prepared by the analyst at a concentration of 0.5 ppb and have an acceptable range of ≥ 0.35 ppb and ≤ 0.65 ppb.

SECTION 3: ITEMS NEEDED FOR ANALYSIS ACCORDING TO METHOD 546

- ABRAXIS® Microcystins/Nodularins-ADDA kit, PN 520011, PN 520011OH, or PN 520011SAES
- Microplate reader, PN 475010 (recommended)
- Reader software for microplate reader (recommended)
- ABRAXIS® Method 546 Accessory Pack, PN 520013
    - Includes Method 546 QCS, Low-CV, and 50 ppb MC-LR Spiking Solution
    - Each accessory pack provides the necessary volume of reagents for use with one ABRAXIS® Microcystins/Nodularins-ADDA ELISA kit (i.e. two partial plate sample analysis batches, one entire plate sample analysis batch, or one IDC analysis batch)
- 20-200 µL micropipettor, PN 704058
- 8-channel pipette, PN 704045
- 10-200 µL pipette tips, PN 300002
- 5 mL serological pipettes, PN 704065
- Serological pipette bulb, PN 704060
- Graduated cylinder capable of measuring 200 mL of volume, PN 705056
- Liquid reservoir basins, PN 704028

- Amber borosilicate sample vials (with caps with PTFE-lined septa):
    - 20 mL, PN 701029
    - 40 mL, PN 701027
    - 250 mL, PN 701030
- 5 cc syringes, glass or plastic (plastic: PN 704013)
- Glass fiber filters, 1.2 µm (PN 706049) or 0.45 µm (PN 706051)
- 10 mg sodium thiosulfate tablets
- DPD test kit for residual chlorine or equivalent
- Deionized or distilled water
- Optional:
    - Electronic repeating pipette, PN 704051
    - 5 mL repeater pipette tips, PN 704038
    - Vortex mixer, PN 300805
    - Cyanotoxin Automated Assay System (CAAS Cube), PN 475006

SECTION 4: COLLECTION/PRESERVATION PROCEDURES

4.1 General Guidelines for Sample Collection

- Do not use ascorbic acid in place of sodium thiosulfate. This is **prohibited**, as the use of ascorbic acid may degrade the toxin, producing inaccurate results.
- For drinking water samples, allow the tap to run for approximately 5 minutes before collection.
- Immediately following collection, invert samples several times until all sodium thiosulfate is dissolved.
- Although ambient water samples do not require dechlorination/quenching, the methods described below to prepare bottles for drinking water collection may be used for ambient water if desired.
- If samples are to be shipped to an external laboratory for analysis, they must be shipped on ice. Proper precautions must be taken to ensure that samples do not reach temperatures greater than 10°C between time of collection and arrival at the testing facility.

4.2 Preparation of Sample Collection Bottles and Sample Collection

Drinking water samples must be treated with **solid** sodium thiosulfate at the time of collection. Liquid solutions of sodium thiosulfate cannot be used, and solid sodium thiosulfate cannot be diluted prior to use. Sample bottles may be prepared in bulk, prior to collection, according to the following procedure:

1. Measure 100 mL of distilled or deionized water (DI H_2O) and pour into a 250 mL amber bottle.
2. Draw a line on the bottle corresponding to the 100 mL with a black marker.
3. Graduate sample bottles by placing each bottle next to the marked bottle and draw a line on each sample bottle corresponding to the 100 mL mark on the original bottle.
4. Add a 10 mg sodium thiosulfate tablet to each newly graduated bottle.

Alternatively, 125 mL sample bottles may be used. Bottles would then be prepared by adding a 10 mg sodium thiosulfate tablet to each bottle and filling to capacity in the field. Note: Freezing samples in glass containers which are filled to capacity will result in bottle breakage. To avoid breakage of sample

collection bottle and loss of sample, samples collected in this manner should be thoroughly mixed, then split into a second glass container before freezing.

Immediately after sampling, shake sample until sodium thiosulfate tablet is completely dissolved.

4.3 Upon Receipt of Samples

- Sample temperature must be verified at the time of receipt to determine whether the 10°C temperature limit has been exceeded. This limit may be exceeded only if the transportation time was too short to allow the samples to chill to 10°C, AND the accompanying ice packs remain frozen. If the 10°C temperature limit has been exceeded and ice packs have melted, samples must be discarded and re-sampled.
- Since chlorine will degrade microcystins, causing false negatives, one sample from each cooler used during shipping/transportation must be tested for residual chlorine. The N,N-diethyl-p-phenylenediamine (DPD)-colorimetric technique, or equivalent, should be used. Residual chlorine levels must be less than the limit of detection for the chosen chlorine assay. A duplicate sample may be collected for residual chlorine testing.
- Freeze samples immediately upon arrival at the testing laboratory, unless they are to undergo immediate lysing/filtering and analysis. If preparing samples to undergo lysing and filtering, see 4.5 Lysing by Freeze/Thaw Procedure for the correct procedure. Freeze the remaining bulk samples.
- If bottles are filled to a volume that is more than half of the bottle capacity during sample collection, the analyst must split the sample into a second glass container to avoid breakage during freezing.

4.4 Sample Hold Time

Analyze samples as soon as possible upon receipt. If samples cannot be analyzed on the date of collection, samples which have been properly collected, quenched, frozen, and stored may be analyzed up to 14 days after collection (see 4.5 Lysing by Freeze/Thaw Procedure).

4.5 Lysing by Freeze/Thaw Procedure

1. Invert the sample repeatedly to thoroughly mix.
2. Pour 5-10 mL of sample into a 20 mL sample vial.
3. Place 20 mL vial on its side in freezer (placing the vial on its side will decrease the potential for breakage of vials during freezing).
4. Thaw sample in a water bath (approximately 35°C).
5. Repeat steps 3 and 4 twice for a total of three freeze/thaw cycles. If the sample was stored frozen, only two additional freeze/thaw cycles will be required.

SECTION 5: EXPLANATION OF “ANALYSIS BATCH” AND REQUIREMENTS

In accordance with EPA Method 546, laboratories monitoring water sources for total microcystins and nodularins will be required to include specific “quality control elements” (QCEs) in each “analysis batch.” An analysis batch must always include the following (See SECTION 2: GLOSSARY OF TERMS for an explanation of each item):

- Calibrators (provided in the test kit)
- 546 Quality Control Sample (546 QCS)
- Laboratory Reagent Blanks (LRB)
- Low Range Calibration Verification Standard (Low-CV)
- Laboratory Fortified Blanks (LFB)
- Samples
- Laboratory Fortified Sample Matrix (LFSM) and Laboratory Fortified Sample Matrix Duplicate (LFSMD)

SECTION 6: PREPARATION OF ANALYSIS BATCH QUALITY CONTROL ELEMENTS (QCEs)

6.1 Laboratory Reagent Blank (LRB)

Use deionized or distilled water treated with sodium thiosulfate (for example, one 10 mg tablet per 100 mL of DI H_2O) if drinking water samples are to be analyzed in a batch. If analyzing only ambient water which was not treated with sodium thiosulfate at time of collection, use untreated DI H_2O (do not add sodium thiosulfate, as the LRB must match the matrix of the samples being tested).

1. Add 10 mL of DI H_2O into two separate 20 mL sample vials.
2. Complete three freeze thaw cycles (as described in 4.5 Lysing by Freeze/Thaw Procedure).
3. Filter the LRB using a 5 cc syringe and syringe filter.
4. The LRB will be analyzed twice, on opposite sides of the plate (once at the beginning with the other QCEs and again at the end of the plate, after the samples). Please see example plate map (TABLE 1: EXAMPLE ANALYSIS BATCH PLATE MAP) for correct positioning.

6.2 Low Range Calibration Verification (Low-CV)

Do not freeze/thaw or filter the Low-CV standard.

The Low-CV may be obtained from Gold Standard Diagnostics Horsham or, alternatively, may be prepared by the testing facility according to the following procedure:

1. Add 10 mL of DI H_2O into a 20 mL sample vial.
2. Add 60 µL of the ABRAXIS® 50 ppb spiking solution to the DI H_2O.
3. Vortex or invert the sample **repeatedly** for 30 seconds to thoroughly mix.
4. Analyze once with the analysis batch. Please see example plate map (TABLE 1: EXAMPLE ANALYSIS BATCH PLATE MAP) for correct positioning.

6.3 Laboratory Fortified Blank (LFB)

DI H_2O treated with sodium thiosulfate (for example, one 10 mg tablet per 100 mL of DI H_2O) must be used if drinking water samples are to be included in an analysis batch. If analyzing ambient water samples only, do not add sodium thiosulfate.

1. Add 10 mL of DI H_2O into two separate 20 mL sample vials.
2. Pipette 120 µL of ABRAXIS® 50 ppb spiking solution into each sample vial.
3. Vortex or invert the sample **repeatedly** for 30 seconds to thoroughly mix.

4. Complete three freeze/thaw cycles, as described in Section 4.5, Lysing by Freeze/Thaw Procedure.
5. Filter each LFB separately using a 5 cc syringe and syringe filter.
6. Analyze each sample vial once on the analysis batch. Please see example plate map (TABLE 1: EXAMPLE ANALYSIS BATCH PLATE MAP) for correct positioning.

6.4 Laboratory Fortified Sample Matrix (LFSM) and LFSM Duplicate (LFSMD)

If running both raw water and drinking water, one set of LFSM/LFSMD is required for each type of sample. If running more than twenty of either type of sample, a second, representative set of LFSM/LFSMD is required for that sample type.

6.4.1 For Drinking Water:

1. Select a new or previously analyzed quenched (treated with sodium thiosulfate) sample.
2. Mix sample well by inverting several times, then measure out 10 mL into two separate 20 mL sample vials.
3. Pipette 100 µL of the ABRAXIS® 50 ppb spiking solution into each vial.
4. Vortex or invert the sample **repeatedly** for 30 seconds to thoroughly mix.
5. Complete three freeze/thaw cycles, as described in Section 4.5, Lysing by Freeze/Thaw Procedure.
6. Filter each vial separately using 5 cc syringes and syringe filters.
7. Analyze both sample vials in the analysis batch. Please see example plate map (TABLE 1: EXAMPLE ANALYSIS BATCH PLATE MAP) for correct positioning.

6.4.2 For Ambient Water:

1. Select a new or previously analyzed sample.
2. Mix sample well by inverting several times, then measure out 10 mL into two separate 20 mL sample vials.
3. Pipette 200 µL of the ABRAXIS® 50 ppb spiking solution into each vial.
4. Vortex or invert the sample **repeatedly** for 30 seconds to thoroughly mix.
5. Complete three freeze/thaw cycles, as described in Section 4.5, Lysing by Freeze/Thaw Procedure.
6. Filter each vial separately using 5 cc syringes and syringe filters.
7. Analyze both sample vials with the analysis batch. Please see example plate map (TABLE 1: EXAMPLE ANALYSIS BATCH PLATE MAP) for correct positioning.

SECTION 7: ANALYSIS BATCH ACCEPTANCE CRITERIA

7.1 Calibration Curve

- %CV of absorbance for each calibration standard must be ≤ 10%. One standard %CV may be ≥ 10% and ≤ 15%, but only if all other standard %CVs are ≤ 10%.
- The r^2 value for the curve must be ≥ 0.980.

If the calibration curve fails either of these requirements, the entire Analysis Batch is invalid and cannot be used. Samples must then be reanalyzed in a new Analysis Batch. If a new Analysis Batch cannot be analyzed on the same day, freeze all samples until they can be re-analyzed. Samples may be stored frozen for up to 14 days after collection.

7.2 Samples

- %CV of absorbance for each sample must be ≤ 15%.

If a sample fails this requirement, it must be reanalyzed with a new Analysis Batch.

7.3 LRB

- Concentration of the LRB must be less than one half of the MRL (MRL = 0.3 ppb).

If the concentration of the LRB is greater than or equal to 0.15 ppb, any positive samples in the Analysis Batch must be reanalyzed. Negative samples are not affected by this requirement and may be reported.

7.4 Low-CV

- Concentration of Low-CV must be ≥ 0.15 ppb ≤ 0.45 ppb.

If the concentration of the Low-CV is outside of this range, the entire Analysis Batch is invalid and cannot be used. All QCEs must be discarded and prepared again for a new Analysis Batch. Sample results may not be reported.

7.5 LFB

- Concentrations of the LFBs must be ≥ 0.36 ppb and ≤ 0.84 ppb.

If the concentration of the LFB is outside of this range, the entire Analysis Batch is invalid and cannot be used. All QCEs must be discarded and prepared again for a new Analysis Batch. Sample results may not be reported.

7.6 LFSM and LFSMD

Calculate the mean percent recovery (%R) for each LFSM and LFSMD set using the equation:

$$\%R = \frac{(A - B)}{C} \times 100\%$$

Where,
A = mean measured concentration of the LFSM and LFSMD
B = measured concentration in the original, unspiked sample
C = fortification concentration (0.5 ppb for drinking water, 1.0 ppb for ambient water)

Subtract the unfortified sample recovery (B) from the mean fortified spiked recovery (A) even if the concentration in the unfortified sample is less than the MRL (0.3 ppb).

Calculate the relative percent difference (RPD) between the LFSM and LFSMD using the following equation:

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100\%$$

Acceptance Criteria

- The %R for the LFSM and LFSMD must be ≥ 60% and ≤ 140%.
- The RPD for the LFSM and LFSMD should be ≤ 40%.

If the first criterion fails, but the criteria for the LFBs were acceptable, then the recovery may be matrix biased. Qualify the result for the sample from which the LFSM/LFSMD was prepared as "suspect-matrix." Likewise, if the second criterion fails, but the criteria for the LFBs were acceptable, then the precision may be matrix biased. Qualify the result for the sample from which the LFSM/LFSMD was prepared as "suspect-matrix."

SECTION 8: PREPARATION OF THE INITIAL DEMONSTRATION OF CAPABILITY (IDC) COMPONENTS

Before field samples can be analyzed in accordance with EPA Method 546, an IDC must be performed by the laboratory to prove accurate determination of microcystins and nodularins by ADDA ELISA.

There are four sections necessary to completing the IDC: demonstration of precision and accuracy, determination of acceptable system background, a Minimum Reporting Limit (MRL) confirmation, and analysis of a quality control sample. Gold Standard Diagnostics Horsham recommends analyzing these four components on a single plate to reduce the number of wells consumed by running the assay multiple times. In addition to the control elements required for the IDC, Gold Standard Diagnostics Horsham also recommends analyzing two additional LFBs and a Low-CV. See TABLE 2: EXAMPLE IDC ANALYSIS BATCH PLATE MAP for correct plate positioning.

8.1 Preparation of sodium thiosulfate treated water

1. Add 200 mL of DI H_2O into an appropriate glass container.
2. Add two 10 mg tablets of sodium thiosulfate to DI H_2O.
3. Mix well by inversion until dissolved.

NOTE: Discard any remaining DI H_2O treated with sodium thiosulfate prepared for the IDC once it has been completed.

8.2 Demonstration of Precision and Accuracy

1. Add 10 mL aliquots of the sodium thiosulfate treated DI H_2O to seven separate 20 mL sample vials.
2. Using a micropipettor, add 100 µL of the ABRAXIS® 50 ppb spiking solution to each vial.
3. Vortex or invert each sample **repeatedly** for 30 seconds to thoroughly mix.
4. Complete three freeze/thaw cycles as described in Section 4.5, Lysing by Freeze/Thaw Procedure.
5. Filter each sample into a 4 mL glass sample vial using 5 cc syringes and syringe filters.

6. Analyze on an Analysis Batch (see TABLE 2: EXAMPLE IDC ANALYSIS BATCH PLATE MAP for correct plate positioning).

8.3 Demonstration of Acceptable Sample Background

1. Add 5 mL of sodium thiosulfate treated DI H_2O into five separate 20 mL sample vials.
2. Complete three freeze/thaw cycles on each bottle as described in Section 4.5, Lysing by Freeze/Thaw Procedure.
3. Filter each sample into a 4 mL glass sample vial using 5 cc syringes and syringe filters.
4. Analyze on an Analysis Batch (see TABLE 2: EXAMPLE IDC ANALYSIS BATCH PLATE MAP for correct plate positioning).

8.4 Preparation of the IDC MRL Samples

1. Add 10 mL of sodium thiosulfate treated DI H_2O into seven separate 20 mL sample vials.
2. Using a micropipettor, add 60 µL of the ABRAXIS® 50 ppb spiking solution to each vial.
3. Vortex or invert the sample **repeatedly** for 30 seconds to thoroughly mix.
4. Complete three freeze/thaw cycles Section 4.5, Lysing by Freeze/Thaw Procedure.
5. Filter each sample into a 4 mL glass sample vial using 5 cc syringes and syringe filters.
6. Analyze on an Analysis Batch (See TABLE 2: EXAMPLE IDC ANALYSIS BATCH PLATE MAP for correct plate positioning).

8.5 Method 546 Quality Control Sample (546 QCS)

Analyze the 546 QCS provided in the ABRAXIS® Method 546 Accessory Pack with the Analysis Batch. No additional preparation is necessary. **Do not add sodium thiosulfate, lyse or filter the prepared 546 QCS.** A 546 QCS must be analyzed during each IDC, and whenever a new lot of calibration standards are used.

SECTION 9: EVALUATION OF IDC RESULTS

9.1 Evaluation of Precision and Accuracy

- The % Relative Standard Deviation (%RSD) between the seven sets of well duplicates must be ≤ 15%.
- The mean recovery for the seven replicates must be ≥ 70% and ≤ 130% of the target value, or ≥ 0.35 and ≤ 0.65 ppb. A macro for the calculation of recoveries and %RSD can be obtained from Gold Standard Diagnostics Horsham upon request.

If the precision and accuracy component of the IDC fails either of these requirements, the precision and accuracy component must be repeated with a set of calibration standards and newly prepared Precision and Accuracy standards (Section 8.1), as well as a Low-CV and Acceptable Sample Background controls (below), even if they passed on a previous Analysis Batch.

Note: Re-analysis of acceptable sample background, IDC MRL, and 546 QCS are not required if these components meet their respective acceptance criteria.

9.2 Evaluation of Acceptable Sample Background

- The result for each of the five LRBs must be less than one-half the concentration of the MRL, or ≤ 0.15 ppb.

If the acceptable sample background component of the IDC fails this requirement, the entire IDC must be repeated using newly prepared controls for each of the categories (Sections 8.1, 8.2, and 8.3).

9.3 Evaluation of Acceptable MRL Sample

- The Upper PIR Limit must be ≤ 150% and the Lower PIR Limit must be ≥ 50%. A macro for the calculation of recoveries can be obtained from Gold Standard Diagnostics Horsham upon request.

If the MRL component of the IDC fails this requirement, the MRL must be repeated with a set of calibration standards, a set of LFBs, a Low-CV, and newly prepared MRL standards (Section 8.3).

Note: Re-analysis of precision and accuracy, acceptable sample background, and 546 QCS are not required if these components meet their respective acceptance criteria.

9.4 Evaluation of Acceptable 546 QCS

- The 546 QCS recovery must be ≥ 70% and ≤ 130% of the target value, or ≥ 0.42 and ≤ 0.78 ppb. A macro for the calculation of recoveries can be obtained from Gold Standard Diagnostics Horsham upon request.

If the 546 QCS component of the IDC fails this requirement, the entire IDC must be repeated using newly prepared controls for each of the categories (Sections 8.1, 8.2, and 8.3).

SECTION 10: PERFORMING METHOD 546 ANALYSIS WITH THE GOLD STANDARD DIAGNOSTICS HORSHAM ABRAXIS® CYANOTOXIN AUTOMATED ASSAY SYSTEM (CAAS CUBE)

Gold Standard Diagnostics Horsham offers an automated instrument for sample analysis using ABRAXIS® Microcystins ADDA kits. The instrument is known as the Cyanotoxin Automated Assay System (CAAS Cube). The instrument is capable of Method 546 analysis, both the Analysis Batch as well as the IDC. For further information about the CAAS Cube, please contact Gold Standard Diagnostics Horsham using the contact information at the end of this document.

TABLE 1: EXAMPLE ANALYSIS BATCH PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 0 0.00 ppb	Std. 4 2.0 ppb	LFB 1 0.60 ppb	S2	S6	S10	S14	LFSMD	S20	S24	S28	S32
B	Std. 0 0.00 ppb	Std. 4 2.0 ppb	LFB 1 0.60 ppb	S2	S6	S10	S14	LFSMD	S20	S24	S28	S32
C	Std. 1 0.15 ppb	Std. 5 5.0 ppb	LFSM	S3	S7	S11	S15	S17	S21	S25	S29	S33
D	Std. 1 0.15 ppb	Std. 5 5.0 ppb	LFSM	S3	S7	S11	S15	S17	S21	S25	S29	S33
E	Std. 2 0.40 ppb	LRB 1 < 0.15 ppb	LFSMD	S4	S8	S12	S16	S18	S22	S26	S30	LFB 2 0.60 ppb
F	Std. 2 0.40 ppb	LRB 1 < 0.15 ppb	LFSMD	S4	S8	S12	S16	S18	S22	S26	S30	LFB 2 0.60 ppb
G	Std. 3 1.0 ppb	Low-CV 0.30 ppb	S1	S5	S9	S13	LFSM	S19	S23	S27	S31	LRB 2 < 0.15 ppb
H	Std. 3 1.0 ppb	Low-CV 0.30 ppb	S1	S5	S9	S13	LFSM	S19	S23	S27	S32	LRB 2 < 0.15 ppb

NOTES:

- **Additional sets of LFSM/LFSMD may be required depending upon the presence of both ambient and drinking water or if greater than 20 samples of either type are analyzed.**
- **A 546 Quality Control Sample (546 QCS) must be analyzed with each new lot of calibrators. Place it after the first set of LFSM/LFSMD at the beginning of the plate. In this example, replace both S1 replicates with the Method 546 QCS.**
- **The plate map above shows the analysis of an entire microtiter plate. As the number of samples on a plate increases, the potential for bias due to unequal incubation periods, otherwise known as drift, increases. To avoid the potential for drift, ensure that the addition of antibody, conjugate, color, and stop are completed within two minutes. If any of these steps cannot be completed in less than two minutes, reduce the number of samples being analyzed on a single plate accordingly. To increase the speed at which the addition of these solutions is performed, a repeater pipette, 8 channel pipette, or an automated analyzer (CAAS Cube) can be used to load these reagents.**

TABLE 2: EXAMPLE IDC ANALYSIS BATCH PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 0	Std. 4	QCS	ASB LRB2	P&A3	IDC MRL4	P&A6	LFB 2				
B	Std. 0	Std. 4	QCS	ASB LRB2	P&A3	IDC MRL4	P&A6	LFB 2				
C	Std. 1	Std. 5	ASB LRB1	P&A2	IDC MRL3	ASB LRB5	IDC MRL6					
D	Std. 1	Std. 5	ASB LRB1	P&A2	IDC MRL3	ASB LRB5	IDC MRL6					
E	Std. 2	Low-CV	P&A1	IDC MRL2	ASB LRB4	P&A5	P&A7					
F	Std. 2	Low-CV	P&A1	IDC MRL2	ASB LRB4	P&A5	P&A7					
G	Std. 3	LFB 1	IDC MRL1	ASB LRB3	P&A4	IDC MRL5	IDC MRL7					
H	Std. 3	LFB 1	IDC MRL1	ASB LRB3	P&A4	IDC MRL5	IDC MRL7					

Key:

ASB = Acceptable Sample Background LRBs, target value ≤ 0.15 ppb

P&A = Precision and Accuracy Samples, target value 0.50 ppb

IDC MRL = Initial Demonstration of Capability Minimum Reporting Level Sample, target value 0.30 ppb

SECTION 11: ASSISTANCE

For ordering or technical assistance, please contact Gold Standard Diagnostics Horsham at (215) 357-3911 or by email at info.abraxis@us.goldstandarddiagnostics.com.

SECTION 12: REFERENCES

U.S. EPA. August 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA Enzyme-Linked Immunosorbent Assay.

For ordering or technical assistance contact:
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