Abraxis Method 546 Guide

Analysis Batch Controls

Laboratory Reagent Blank

- Description: Control that is used to determine if any interferences are introduced during analysis. It is an aliquot of reagent water that is lysed and filtered using the same procedure used for sample processing
- Preparation Guidelines
  - If drinking water samples are being analyzed with the batch, use DI/Distilled water treated appropriately with sodium thiosulfate
  - If only testing raw water, do not treat DI/Distilled water with sodium thiosulfate
- Location on plate: LRB will be analyzed twice, once immediately after the standards and once at the end of the plate.
- Acceptable range: Must be ≤ 0.15 ppb (less than one half the MRL)

Analysis Batch Controls

Laboratory Reagent Blank: Preparation

Measure 10 mL of DI H_2O into two 20 mL vials

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Three Freeze/Thaw Cycles

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Filter each separately with glass fiber filters

Analysis Batch Controls

Low-CV

- Description: Control that is at a concentration that is less than or equal to the MRL (0.3 ppb) which is used to confirm the accuracy of the standard curve near the MRL.
- Preparation Guidelines
  - Do not freeze/thaw or filter the Low-CV
- Preparation:
  - May be obtained by Abraxis in the Method 546 Accessory Pack
- Location on plate: Low-CV will be analyzed once, immediately after the first LRB
- Acceptable range: Must be ≥ 0.15 or ≤ 0.45 ppb

Analysis Batch Controls

Low-CV: Preparation

Measure 10 mL of DI H_2O into a single 20 mL vial

Add 60 uL of the Abraxis 50 ppb standard into the vial

Vortex or invert repeatedly to mix

DO NOT FREEZE/THAW OR FILTER THE LOW-CV

Analysis Batch Controls

Laboratory Fortified Blank

- Description: An aliquot of reagent water to which a known concentration (0.6 ppb) is added to verify method performance in the absence of sample matrix.
- Preparation Guidelines
  - If finished drinking water samples are included in the batch, properly quenched DI H_2O must be used to prepare the LFB. If only analyzing raw drinking water, do not quench the DI H_2O.
- Location on plate: Immediately after the Low-CV and again at the end of the plate immediately after the last sample.
- Acceptable range: Must be ≥ 0.36 or ≤ 0.84 ppb

Analysis Batch Controls

Laboratory Fortified Blank: Preparation

Measure 10 mL of DI H_2O into two separate 20 mL vials

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Pipette 120 uL of the Abraxis 50 ppb standard into each sample vial

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Three Freeze/Thaw Cycles

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Filter each separately with glass fiber filters

Analysis Batch Controls

Laboratory Fortified Sample Matrix and LFSM Duplicate

- Description: Aliquot of a new or previously analyzed sample to which a known concentration of microcystins is added. It is used to determine if the sample matrix contributes bias to the results.
- Preparation guidelines:
  - If analyzing only drinking water, a quenched sample must be used to prepare the LFSM and LFSMD.
  - If only analyzing raw water, a non-quenched sample must be used to prepare the LFSM and LFSMD.
  - If analyzing both raw and finished drinking water, a representative set of LFSM and LFSMDs must accompany the samples.
  - If more than 20 samples are to be analyzed of either type, a second representative set of LFSM and LFSMD must be analyzed.
- Location on plate: Immediately after the first LFB. If analyzing more than one set of LFSM and LFSMD, the second set will follow half of the samples.
- Acceptable range:

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 unfortified 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:

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

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

Analysis Batch Controls

LFSM and LFSMD Acceptability Criteria

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 unfortified 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:

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

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

Analysis Batch Controls
LFSM/LFSMD: Preparation (Drinking Water)

Select a new or previously quenched sample, invert several times to mix and measure 10 mL into two separate 20 mL sample vials

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Pipette 100 uL of the Abraxis 50 ppb standard into each sample vial, and vortex or invert repeatedly to mix

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Three Freeze/Thaw Cycles

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Filter each separately with glass fiber filters

Analysis Batch Controls
LFSM/LFSMD: Preparation (Raw Water)

Select a new or previously analyzed sample, invert several times to mix and measure 10 mL into two separate 20 mL sample vials

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Pipette 200 uL of the Abraxis 50 ppb standard into each sample vial, and vortex or invert repeatedly to mix

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Three Freeze/Thaw Cycles

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Filter each separately with glass fiber filters

Analysis Batch Sample Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 0	Std. 4	LFB 1	S2	S6	S10	S14	LFSMD	S20	S24	S28	S32
	0.00 ppb	2.0 ppb	0.60 ppb									
B	Std. 0	Std. 4	LFB 1	S2	S6	S10	S14	LFSMD	S20	S24	S28	S32
	0.00 ppb	2.0 ppb	0.60 ppb									
C	Std. 1	Std. 5	LFSM	S3	S7	S11	S15	S17	S21	S25	S29	S33
	0.15 ppb	5.0 ppb										
D	Std. 1	Std. 5	LFSM	S3	S7	S11	S15	S17	S21	S25	S29	S33
	0.15 ppb	5.0 ppb										
E	Std. 2	LRB 1	LFSMD	S4	S8	S12	S16	S18	S22	S26	S30	LFB 2
	0.40 ppb	< 0.15 ppb										0.60 ppb
F	Std. 2	LRB 1	LFSMD	S4	S8	S12	S16	S18	S22	S26	S30	LFB 2
	0.40 ppb	< 0.15 ppb										0.60 ppb
G	Std. 3	Low-CV	S1	S5	S9	S13	LFSM	S19	S23	S27	S31	LRB 2
	1.0 ppb	0.30 ppb										< 0.15 ppb
H	Std. 3	Low-CV	S1	S5	S9	S13	LFSM	S19	S23	S27	S32	LRB 2
	1.0 ppb	0.30 ppb										< 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 are performed, a repeater pipette, 8 channel pipette or an automated analyzer (CAAS) can be used to load these reagents.**

Initial Demonstration of Capability Controls
Acceptable Sample Background

- Preparation guidelines:
  - Use a portion of the remaining DI H_2O treated with sodium thiosulfate prepared for the demonstration of precision and accuracy to prepare Acceptable Sample Background controls
- Location on plate: scattered throughout plate, first one after the Method 546 QCS
- Acceptability criteria:
  - The result for each of the five LRBs must be less than one-half the concentration of the MRL, or ≤ 0.15 ppb.

Initial Demonstration of Capability Controls

Acceptable Sample Background: Preparation

Aliquot 5 mL of quenched DI H_2O into five separate sample 20 mL sample vials

Three Freeze/Thaw Cycles

Filter each separately with glass fiber filters

Initial Demonstration of Capability Controls

Precision and Accuracy

- Location on plate: Scattered throughout plate after the first Acceptable Sample Background control
- Acceptability Criteria:
  - 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 Abraxis upon request.

Initial Demonstration of Capability Controls

Precision and Accuracy: Preparation

Measure 200 mL in an appropriate glass container and add two 10 mg tablets of sodium thiosulfate, mix well by inversion to dissolve

Aliquot 10 ml of the above solution into seven separate 20 mL vials, along with 100 uL of the Abraxis 50 ppb standard. Mix well by inversion or vortex.

Complete 3 Freeze/Thaw cycles, and use a glass fiber filter to filter each one in a separate vial

Initial Demonstration of Capability Controls

IDC MRL

- Preparation guidelines:
  - Use a portion of the remaining DI H_2O treated with sodium thiosulfate prepared for the demonstration of precision and accuracy to prepare IDC MRLs
- Location: scattered throughout plate, first one after the first Acceptable Sample Background control
- Acceptability criteria:
  - 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 Abraxis upon request.

Initial Demonstration of Capability Controls
IDC MRL

Aliquot 10 mL of treated DI H_2O into seven separate 20 mL vials

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Pipette 60 uL of the Abraxis 50 ppb standard into each sample vial, and vortex or invert repeatedly to mix

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Three Freeze/Thaw Cycles

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Filter each separately with glass fiber filters

Method 546 QCS

- Analyze a Method 546 QCS, which is contained within the Abraxis Method 546 Accessory Kit, each time an IDC is attempted or when a new lot of calibration standards is used.
- Analyze the Method 546 QCS provided in the Abraxis 546 Accessory Pack with the IDC/Analysis Batch. No additional preparation is necessary. **Do not use the QCS contained in the base Microcystins kit (PNs 520011 and 520011OH).**
- Do not add sodium thiosulfate, lyse or filter the 546 QCS

Initial Demonstration of Capability Sample Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 0	Std. 4	546 QCS	ASB LRB2	P&A3	IDC MRL4	P&A6	LFB 2				
B	Std. 0	Std. 4	546 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

Questions or Comments?

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