MQOs[i] for Determination of Cyanotoxins[ii] in Water and Tissue Samples by Ligand-Binding Assays (by ELISA and RBA)

[ii] *MQOs developed specifically for determination of microcystins, anatoxin-a, and nodularin (toxins produced by cyanobacteria)*

Table 1. Lab Quality Control for Microcystins, Anatoxin-a, and Nodularin in Fresh Water and Tissue Samples by Ligand-Binding Assays

Lab Quality Control	Frequency of Analysis	Measurement Quality Objective	*DQ Indicator or Reasoning*
Calibration (not reported to SWAMP database)	Initial method setup and develop calibration curve for each microtiter plate	• R >0.990 or r^2 >0.980 (10) • RSD ≤15% for well duplicates based on absorbance OR • RSD ≤30% for well duplicates based on concentration • Minimum 5 points run in duplicate wells, arithmetic mean used for calibration result • At least 1 calibration point at or below RL	Bias, Instrument QC
Background absorbance/ Instrument zero (not reported to SWAMP database)	Per microtiter plate or 20 environmental samples for non-microtiter plate method[1]	Result <Method detection limit	Instrument QC
Matrix spike [2]	Per microtiter plate or 20 environmental samples for non-microtiter plate method	Recovery 50-150% of true value	Matrix accuracy
Laboratory positive control	Per microtiter plate or 20 environmental samples for non-microtiter plate method	• Recovery 70-130% of true value • RSD ≤20% for well duplicates (triplicate recommended), report result from each replicate [3]	Lab accuracy and precision
Environmental Sample Duplicate *OR* Matrix Spike Duplicate [4]	Per microtiter plate or 20 environmental samples for non-microtiter plate method	• Environmental sample duplicate: RSD ≤20% for well duplicates (triplicate recommended), report result from each replicate [3] • Matrix spike duplicate: RPD ≤25%	Matrix precision
Laboratory blank	Per microtiter plate or 20 environmental samples for non-microtiter plate method	Result <Reporting Limit	Representativeness of analytical system

[1] The term "environmental samples" refers to the unknown samples; thus, quality control samples should not be included when calculating every 20 environmental samples.

[2] If dilution is necessary, the matrix spike sample should be diluted *after* addition of spike solution to the environmental sample.

[3] Calculate the arithmetic mean for minimum of 2 replicates. Report arithmetic mean under the "LabResultComments" field in the SWAMP data entry template.

[4] Performance of the duplicate on the matrix spike is preferred when environmental samples have low or non-detections. If an environmental sample duplicate is used, result of original must be >RL.

[i] These MQOs have been developed for current SWAMP methodology. This does not limit the use of the MQOs for other laboratory methods. Please feel free to contact the OIMA Helpdesk (OIMA-Helpdesk@waterboards.ca.gov) to request assistance to adapt the MQOs for an additional laboratory method.

Table 2. Lab Quality Control Corrective Actions for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Ligand-Binding Assays

Lab Quality Control	Recommended Corrective Action [1]
Calibration	If calibration does not meet acceptance criteria, then verify instrument parameters prior to re-analyzing samples. Indications of poor stability include low OD or poor comparability of analytical results between batches. Confirm expiration dates and manufacturing lot numbers. If using a kit, do not mix kit reagents or plates from different lot numbers in a single analytical batch. [(4)] It is acceptable to use different lots of reference material; for example, to use secondary source standards.
Matrix Spike	Re-analyze samples or plate as appropriate. Examine the recovery obtained for the matrix spike duplicate. Examine the results of the other QC samples (i.e. reference material) to determine if other analytical problems are a potential source of the poor measurements. Prior to re-processing samples, add further processing (e.g. dilution) steps to reduce matrix interference. [(9)]
Matrix Spike Duplicate	Re-analyze samples or plate as appropriate. Examine the recovery obtained for the matrix spike. Examine the results of the other QC samples (i.e. reference material) to determine if other analytical problems are a potential source of poor measurements. Prior to re-processing samples, add further processing steps (e.g. dilution) to reduce matrix interference (if needed). [(9)]
Laboratory Positive Control	If low or high recovery, outside of acceptable range, re-analyze samples or plate as appropriate. Variable results between well replicates may indicate drift or other issue. Likely causes include: poor plate washing technique, prolonged time during plating, inappropriate laboratory temperatures, and low/high temperature of reagents during sample processing. [(4,9)]
Laboratory duplicate	Re-analyze samples or plate as appropriate. Review the results of the other QC samples (i.e. other duplicate matrix spike) to determine if other analytical problems are a potential source of poor performance. Investigate preparation of sample for source of variability (e.g. initial pH should be neutral).
Laboratory Blank	If result is between method detection limit (MDL) and reporting limit (RL), then flag batch. If result is ≥RL then re-process samples and reanalyze. Investigate source of contamination. Consider potential contamination by filters used in processing the sample. Recommend testing of each filter manufacturing lot for potential contamination prior to use in routine testing.
Instrument Blank	Verify blanking procedure prior to re-analyzing. Check plate reader functions if OD readings are high and the color was not dark. Common causes of high background include inadequate washing or contamination of reagents. [(4)] Avoid re-using microtiter plates from prior batches unless method utilizes disposable well strips.
Filter Blank	If result is between MDL and RL, then flag batch. If result is ≥RL then re-process samples and reanalyze. Discard filter manufacturing lot and investigate source of contamination. Indications of bacterial contamination include low result and high OD reading.

[1] Documentation should be included when a MQO is not met and appropriate corrective actions are taken. Please include this documentation in the "LabBatchComments" field of the SWAMP data template or in a Corrective Action Report. The documentation should provide justification for excluding the record(s) from the lab batch **or** why the record(s) should be considered in the lab batch after corrective actions.

Table 3. Field Quality Control for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Ligand-Binding Assays

Field Quality Control	Frequency of Analysis	Measurement Quality Objective	*DQ Indicator or Reasoning*
Equipment blank [1]	5% of total project samples	<Reporting Limit	Field Process Bias
Field duplicate	5% of total project samples	RPD ≤25%	Sample Collection Precision
Filter blank	5% of total project samples (only required if filtration done in field)	<Reporting Limit	Sample Process Bias

[1] Equipment blank refers to preparing a sample bottle blank or sampling equipment blank. Sample bottle blank is only required if the sample bottle is re-cleaned from prior sampling. Sampling equipment blank is only required if a device (e.g. cup, pump) is used to transfer the environmental sample from water body to the sample container.

Table 4. Field Quality Control Corrective Actions for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Ligand-Binding Assays

Field Quality Control	Recommended Corrective Action [1]
Equipment Blank	Investigate the source of contamination. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged. (6)
Field Duplicate	Visually inspect the sample to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator for further actions. (6)
Filter Blank	Investigate the source of contamination. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged. (6)

[1] Documentation should be included when a MQO is not met and appropriate corrective actions are taken in the field at the time of collection. Please include this documentation in the collection comments found on the field entry form or in a Corrective Action Report. The documentation should provide justification for excluding the record(s) from the data set **or** why the record(s) should be considered in the data set after corrective actions.

Table 5. Sample Handling for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples

Matrix	Container [3,6]	Microcystin & Nodularin Temperature & Holding Time	Anatoxin-a Temperature & Holding Time [4]
Water *(for total toxin [1])*	Amber glass (recommended) or dark colored HDPE [3]	Cool to <6 °C (in dark) for up to 5 days, then freeze at <-20°C. [(2)] Long term storage of up to 6 months at -80°C. [(10,11)]	Cool to <6 °C (in dark) for up to 3 days, then freeze at <-20°C. [(2)] Long term storage of up to 6 months at -80°C. [(10,11)]
Water *(for dissolved phase or filtrate)*	Amber glass (recommended) or dark colored HDPE [3]	Cool to <6 °C (in dark) for up to 48 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(10,11)]	Cool to <6 °C (in dark) for up to 48 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(10,11)]
Water *(for particulate phase [2] or periphyton)*	Amber glass (recommended) or dark colored HDPE [3,5]	Cool to <6 °C (in dark) for up to 24 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(7)]	Cool to <6 °C (in dark) for up to 24 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(7)]
Tissue [7] *(for dissected tissue)*	Amber glass (recommended) or dark colored HDPE	Freeze short term at <-20°C. Long term storage of up to 6 months at -80°C. [(1,5)]	Freeze short term at <-20°C. Long term storage of up to 6 months at -80°C. [(1,5)]

[1] Analysis of intracellular and extracellular cyanotoxins.
[2] Analysis of intracellular cyanotoxins.
[3] Glass containers recommended to prevent adsorption of toxin to plastic material. [(3,8)]
[4] Limit holding time for anatoxin-a analysis to reduce toxin degradation.
[5] Filtering conducted in the field may utilize petri dishes as an alternative container to store filters.
[6] If amber or dark colored containers are not available, foil may be used to cover containers. Ensure foil completely covers container.
[7] Table 5 has been developed for analysis of muscle and organ tissue from fish and shell fish. This does not limit the use of the guidelines for other tissue types. Please feel free to contact the OIMA Helpdesk (OIMA-Helpdesk@waterboards.ca.gov) to request assistance to adapt the guidelines for an alternative sample type.

References

(1) Al-Sammak, M. A, Hoagland K.D., Cassada, D., and Snow, D.D., 2014, Co-occurrence of the Cyanotoxins BMAA, DABA and Anatoxin-a in Nebraska Reservoirs, Fish, and Aquatic Plants, Toxins, Volume 6, 488-508.
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