Quality Control Details

Testing and Measurement Protocols

The following charts summarize the measurement protocols used for biofilm and spore enumeration as well as for water parameter sampling:

Sample Measurements

Parameter	Analytical method	Sampling procedure	Sample size/ container	Replicates	Preservation and storage	Maximum holding time
Heterotrophic Plate Count	*Standard Method 9215A.*	Scraped from 38 cm^2 coupon into large Petri dish	100 mL sample container/ .1 mL spreadplate	3	Initiate analysis immediately, or store at 4ºC.	24 hours
Spread plating	*Standard Method 9215C*	Scraped from 39 cm^2 into 120 mL coliform sample bottle	~.1 mL	3	Initiate analysis immediately, or store at 4ºC.	24 hours

Water Quality Samples

Parameter	Analytical method	Reference	Sample size/ container	Preservation and storage	Maximum holding time
pH	pH-meter	*Standard Methods* 1992	100 mL beaker	NA	NA
Conductivity	Extech handheld conductivity and temperature meter	*Standard Methods 2510 B*	In situ	NA	NA
Temperature	Extech handheld conductivity and temperature meter	*Standard Methods* 2550	In situ	NA	NA
Total and free chlorine	Hach DR 2400 Spectrophotometer	*Standard Methods 4500-CL G.*	50 mL beaker	NA	NA

QA Objectives

All analytical samples taken included positive and negative controls to ensure that the samples that are analyzed are accurately defined. No fewer than three replicates were taken to get representative samples. Samples that are outside of the limits of detection, or that do not meet the acceptance criteria required for the method, will be noted and checked for possible errors contributing to the result. Where possible, the tests will be repeated. The following table summarizes the QA samples to be taken.

QA/QC Criteria

Media	Measurement	QA/QC Check	Acceptance Criteria	Corrective Action	Frequency
Water Parameters		Check Standard	80-120% recovery	Retake samples	
	pH	Check against pH 7 buffer	± 0.2 pH units	Use different DO meter	Every sampling event
	Alkalinity	Negative control samples	6.9<pH<7.1	Recalibrate meter and recheck	When time and resources permig
	Conductivity	Check solutions of 200 mS and 2.00 mS	Reading ± 10% of expected value	Adjust meter	During Phase 2 experiments
	Free and total chlorine	Check tap water fresh from the system	Reading ± 10% of expected value (~1 mg/L)	Calibrate spectrophotometer/ replace reagents	Weekly when coupons are exposed to drinking water
	Temperature	Check thermometer against a calibrated thermometer	±0.5ºC	Note discrepancies	Weekly and during every sampling event
Coupon	Spore Enumeration	TSA Blank: Incubate plates	No observed growth	Remake plates	Every spore sampling event
		Spore Viability and quantitation: Positive Control plate spike (Known quantity of spores spiked onto TSA)	100-200 CFU/plate	Check spore suspension and dilution procedures	Every spore sampling event
		Coupon check: Process coupon without adding spores to determine if there is contamination on the coupon. (this is combined with matrix spike)	No observed growth	Determine potential sources of contamination, sterilize all surfaces and equipment and process a second coupon.	Two times for each surface preparation pipe material combination
		Buffer check: Plate buffer solution on TSA to determine sterility	No observed growth	Remake buffer/autoclave all glassware	Every spore sampling event
		Tap water check: Plate tap water used for growing biofilm, and rinsing/spiking coupons	No observed *B. globigii* growth	Sample tap water from tank a second time and autoclave all glassware	Every spore sampling event
		Matrix spike: Scrape biofilm from iron, cement-lined, and PVC. Spike each with 1 mL of spore suspension to determine impact of corrosion on spore enumeration.	100-200 CFU/plate	Compare to counts without corrosion and note differences	Two replicates for each phase.
		Membrane Filter Sterility check: Sterile PBDW with .01% Tween added to MF UV sterilized funnel and sterile filter.	No observed *B. globigii* growth	Resterilize funnel again, ensure that filters are sterile, clean funnels to ensure there is no rust/corrosion on surfaces.	One check for every 10 samples.
	Biofilm HPC	Buffer blank: Plate buffer solution on R2A plates	No observed growth	Remake buffer	Every biofilm sampling event
		R2A Blank: Incubate plates	No observed growth	Remake plates	Every biofilm sampling event

Equipment

Equipment such as the pH meters were used in accordance with the manufacturer's manuals for those pieces of equipment. Larger equipment (refrigerators, autoclaves) were operated using the standard SOPs outlined in the facility SOP manual. Laboratory equipment and instrumentation were checked for accuracy as directed in Standard Methods 9020 B. The following chart summarizes data requirements for equipment:

Data Requirements

Data Quality Acceptable If…	Corrective Action If Unacceptable
All equipment, sensor, and meter calibrations are current.	Out-of-date calibrations will be corrected by recalibration or replacement of item and the analysis redone.
Reagents used are not past expiration date. Reagent grade chemicals are used. Microbiological grade agar used. Traceable standards. Negative controls are used to test for sterility.	Expired reagents will be replaced and the analysis redone. Reject chemicals/agar that is not proper grade.
Refrigerators, autoclaves, and incubators are operating at the required temperature.	If temperatures were not maintained within the required limits of the test, the equipment will be fixed or calibrated and the analysis will be redone.

The following chart summarizes calibration, use, and maintenance for the equipment to be used:

Calibration, Use, and Maintenance of Equipment

Equipment	QA/QC	Frequency	Acceptable Criteria	Corrective Measures
Centrifuges	EPA centrifuges are under a Preventative Maintenance Agreement (PMA) and undergo an annual PM.	Quarterly or as described	Described in instrument manual or SOP	Described in instrument manual or SOP: All non-conformances are corrected before the instrument is put back to use.
Autoclaves	Autoclaves use tape with each batch that contains the date, time, length of cycle, and contents of the cycle. Autoclaves are under a PMA and undergo a quarterly PM.			
NanoPure water purification system	Check conductivity			
Temperature controlled devices	All water baths, refrigerators, freezers, etc. will be checked prior to and after use.			
Balances	Balances are calibrated monthly and annually serviced through the PMA.			
pH and dissolved oxygen meters	pH meters are calibrated daily in buffer.			
Micropipettes	Micropipettes are calibrated annually.	Annually or as described		
Tracking and assessment of media, reagents and supplies	All medium is purchased from a nationally recognized supplier. Negative and positive controls are included in each batch. Lot, date of receipt, and expiration dates are all recorded in the laboratory logbook for reagents and media.	As needed or when new chemicals are received	Ensure critical information is recorded.	Document if information is available; qualify any results that are suspect based on uncertainties with expiration dates.