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Pilot-Scale Decontamination of Surrogate Radionuclides in a Pilot-Scale Drinking Water Distribution System

Office of Research and Development
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Pilot-Scale Decontamination of Surrogate Radionuclides in a Pilot-Scale Drinking Water Distribution System

U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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Disclaimer

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List of Acronyms

BWS	bulk water sample
DI	deionized
DSS	distribution system simulator
EDTA	ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
HPC	heterotrophic plate count
MPN	most probable number
NHSRC	National Homeland Security Research Center
ORP	oxidation-reduction potential
PVC	polyvinyl chloride
QA	quality assurance
QC	quality control
SOP	Standard Operating Procedure
T&E	Test and Evaluation

Executive Summary

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program conducts research to provide the nation's drinking water utilities with tools that would help them recover from contamination of distribution system infrastructure. Accidental or intentional contamination of water systems could result in contaminated infrastructure that would need remediation before the system was returned to service. This study examined decontamination of non-radioactive cesium, strontium and cobalt in a pilot scale model of a drinking water distribution system. The system was outfitted with coupons (excised pipe wall samples) of corroded iron and cement-mortar, which are common distribution system pipe materials. Results from this study were compared with similar decontamination data generated in smaller bench scale experimental systems. The primary findings are as follows:

- Cesium was not persistent on corroded iron in the pilot scale system, similar to the bench scale studies. These results are consistent with the literature that shows that cesium reversibly adsorbs to iron oxides. The bench scale results suggest that clean water flushing alone would remove cesium from corroded iron. Clean water flushing may be effective at removing adhered cesium from iron, but addition of potassium chloride could enhance the decontamination. Cesium was more persistent on cement-mortar relative to iron, but potassium chloride was an effective decontaminant. Past bench scale data and some of the pilot-scale coupons showed that clean water flushing removed cesium from cement-mortar coupons. Pipe surface characteristics variability can influence contaminant adherence and decontamination effectiveness. If decontamination of cesium from water infrastructure in the field is necessary, the data suggests that flushing and application of potassium chloride are effective decontamination methods. Potassium chloride ions can compete with the cesium on the pipe material surface.

- Cobalt adhered to corroded iron and cement-mortar water infrastructure, and EDTA was an effective decontaminant for both surfaces, a finding which was consistent with past bench scale decontamination data. On the pilot scale, complete dissolution of EDTA in the bulk water phase was a challenge, which resulted in inconsistent levels of decontamination on the coupons. The lack of full EDTA dissolution was attributed to a drop in pH, which affected EDTA solubility. When implementing EDTA decontamination in a real distribution system, addition of a basic compound (i.e., sodium hydroxide) may be necessary for complete dissolution.

- Strontium adhered to both corroded iron and cement-mortar, but the amount of initial persistence on cement-mortar was 20 to 25 time higher than on iron. Ammonium acetate

(0.01 M) did remove the adhered strontium, but not to the extent observed in bench scale studies using 0.2 M. Due to the large amount of ammonium acetate needed to achieve 0.2 M in a real water distribution system, adequate decontamination of adhered strontium may require a physical removal operation (e.g., pigging) in addition to flushing and ammonium acetate injection.

1.0 Introduction

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program conducts research to provide the nation's drinking water utilities with tools that would help them recover from contamination of distribution system infrastructure. Accidental or intentional contamination of water systems could result in contaminated infrastructure that would need remediation before the system was returned to service. Development of strategies to manage contaminated infrastructure requires an understanding of the persistence of radionuclides on drinking water infrastructure and the removal, or decontamination, of the adsorbed radionuclides. Currently, this is a topic with little published data. The purpose of this study is to examine the persistence of surrogate radionuclides on drinking water infrastructure and the decontamination in a pilot scale model of a water distribution system.

A pilot-scale drinking water distribution pipe system was constructed with removable coupons (excised pipe wall samples) of unlined, corroded iron and cement-mortar lined iron, which are common distribution system materials. Contamination and decontamination experiments were conducted by injecting solutions of non-radioactive cesium, cobalt or strontium salts. These salts acted as chemical surrogates for radioactive cesium-137, cobalt-60 and strontium-90. Decontamination was conducted by adding chemical decontaminants followed by flushing with water. The results presented in this report provide data on the effectiveness of decontamination agents and flushing on a pilot-scale. Data from this report are also compared to contamination and decontamination data from smaller bench scale studies. This comparison provides insight into whether data from simpler bench scale studies is scalable to larger, yet more realistic, pilot scale studies.

2.0 Pilot Scale System and Methods

2.1 Testing Overview

During the tests, cesium chloride (99.99% pure, Acros Organics, Thermo Fisher Scientific, Waltham, MA), cobalt chloride (97% pure, anhydrous, Acros Organics), or strontium chloride (99.99% pure, anhydrous, Acros Organics) solutions were introduced into the distribution system simulator (DSS) (described in section 2.2) through the recirculation tank to achieve target "in-

pipe" concentrations between 4 mg/L and 10 mg/L. Subsequent to the completion of the contamination protocol (described later under the "Experimental Protocol" section of the report), decontamination strategies (e.g., flushing, chemical agents) were used to remove the radiological contamination from the bulk liquid phase as well as the DSS pipe walls. The decontamination agents used included: potassium chloride (99% pure, ACS certified, Thermo Fisher Scientific, Waltham, MA), ethylene-diamine-tetraacetic acid (EDTA) (99.5% pure, Thermo Fisher Scientific), and ammonium acetate 98% pure, HPLC grade, Thermo Fisher Scientific). The specific concentration of each contaminant and decontaminant are presented in Tables 4, 5 and 6 in Section 3 (Results).

2.2 Experimental Apparatus

Experiments were conducted at the EPA's Test and Evaluation (T&E) Facility in Cincinnati, Ohio using a DSS pipe loop. Figure 1 depicts a schematic 3-D overview of the DSS. The arrows depict flow during normal operation. The main components of the DSS are a large reservoir to supply water, approximately 75 ft (23 M) of 6 in (15.2 cm) diameter polyvinyl chloride (PVC) interconnected main pipe, a 100 gallon (378.5 L) re-circulation tank (in-line with the main pipe), water pumps, associated valves/fittings and small-diameter interconnecting pipes, and electronic control devices necessary to operate the system. The total volume of the DSS (including the 85 gallons [322 L] in the recirculation tank) is approximately 240 gallons (908 L). The interior surface area of the loop, including the recirculation tank (available for adsorption), is approximately 25,000 in^2 (161,250 cm^2). Operation of the DSS system with the in-line recirculation tank causes an injected contaminant to homogeneously mix within a few minutes in the main pipe. The DSS is also equipped with sensors that continuously measure the basic water quality parameters such as pH, conductivity, temperature, free chlorine and oxidation-reduction potential (ORP).

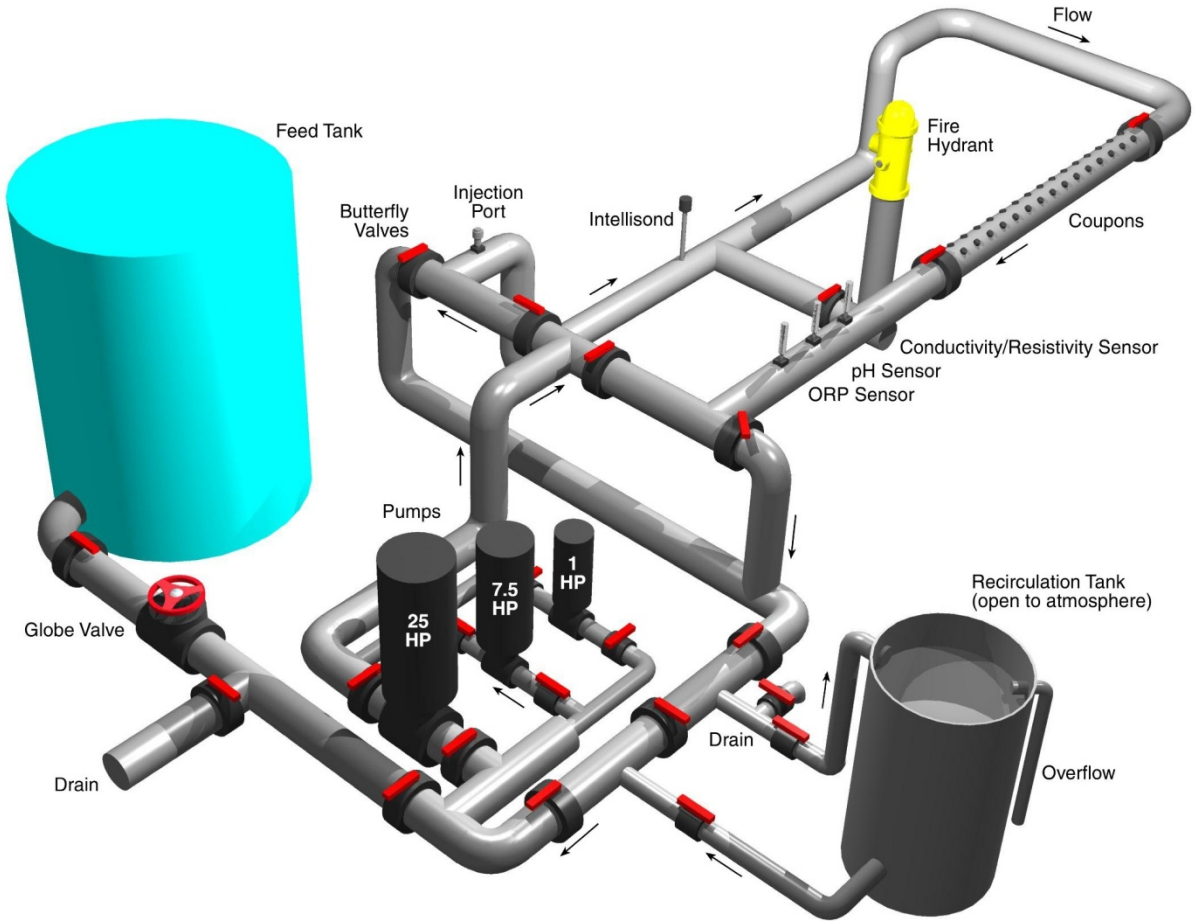

Figure 1: Drinking water distribution system simulator at the EPA T&E facility.

2.3 Experimental Protocol

The test protocol consists of the following steps:

1) Cultivation of Biofilm – Establish background conditions and biofilm
2) Contamination Phase – Contaminant introduction
3) Decontamination Phase – Decontaminant introduction
4) Flushing – Flushing with clean tap water

Online water quality instruments are used to monitor pH, ORP, conductivity, temperature, flow, and pressure during each step. Grab samples for free and total chlorine are collected along with bulk water samples (BWS) and coupon samples as described in the individual step descriptions.

Step 1 - Cultivation of Biofilm:

Cultivation of biofilm in the DSS was accomplished by passing Cincinnati tap water continuously through the DSS and measuring the heterotrophic plate count (HPC) concentration of both the bulk

water and inside pipe surface. The water in the loop was circulated using a centrifugal pump (operating at 88 gallons per minute (gpm) [333 L/min] achieving 1 ft/sec [0.3 m/sec] velocity) to facilitate biofilm formation over a 4-week period. Fresh tap water was added at the rate of 0.8 gpm (6.2 L/min) during the cultivation period to maintain a residual free chlorine concentration in the loop.

A series of 30 coupons (1 in^2 [6.5 cm^2]) with specific test materials (15 ductile iron and 15 cement) set in threaded plugs were inserted into the removable section of the pipe loop (previously shown in Figure 1). The coupons were removed at specific times during the test (i.e., examination of biofilm prior to the test for background, contamination phase, decontamination phase and flushing phase).

Prior to each test, a set of coupons were collected and analyzed for HPC and the selected chemical contaminant. The coupons were collected by shutting off the pump and segregating the removable section of PVC-pipe by closing the flanking butterfly valves, removing the coupons and replacing them with a blank plug. The background HPC/biofilm sample was collected by scraping the coupon surface using a sterilized surgical scalpel. The scraped material was suspended in sterile buffer, homogenized and analyzed for HPC to determine the formation of biofilm on the coupons.

Step 2 – Contamination Phase:

During this step, the radiological surrogate was introduced into the DSS through the recirculation tank to achieve a target concentration between 4 mg/L and 10 mg/L in the loop. A bulk water sample (BWS) was collected after 5 minutes of mixing at 88 gpm (333 L/min). Then, the DSS operating flow rate was reduced and the contaminant was recirculated for 2 hours at 10 gpm (37.9 L/min). After this 2-hour period, the contaminated bulk water was sampled again and a coupon sample set was collected to evaluate the adsorption of chemical onto the pipe surface.

At the end of this step, the DSS was drained and filled with tap water twice to remove as much of the remaining contaminant from the bulk phase as possible.

Step 3 - Decontamination Phase:

The selected chemical decontaminant was introduced into the DSS and re-circulated continuously at 88 gpm (333 L/min) for 20 minutes. After this time, the recirculation pump was turned off, the back-pressure valve was opened, and loop contents were kept static during the rest of the decontamination phase (22 hours). One set of coupons was removed from the DSS at 30, 60, 90, 120, 180, 240, 360, 1,200 and 1,320 minutes. A BWS was collected at each of these coupon sampling times with an additional BWS collected at 15 minutes after the introduction of

decontaminant. A duplicate BWS was collected at 60 minutes. The BWSs were preserved in Nalgene™ (Thermo Fisher Scientific), with 2 drops of concentrated nitric acid.

The coupon removal process was similar to what was described in Step 1. One notable exception is that the scraped material was suspended in deionized (DI) water (instead of sterile buffer), homogenized and analyzed for metals. At the end of this step, the DSS was drained and filled with tap water twice to remove the decontaminant present in the bulk phase.

Step 4 – Flushing:

The tap water-filled loop was recirculated at approximately 88 gpm (333 L/min) (~1 ft/sec [~0.30 m/sec]) with a fresh water influx at a 10 gpm (37.9 L/min) rate to provide the flushing action. Coupon samples were removed at 120, 240 and 1,320 minutes after the 10 gpm (37.9 L/min) flushing action is initiated. A duplicate coupon sample was collected at 120 minutes. The coupon removal process was similar to what was described in Step 3. The scraped material was suspended in DI water, homogenized and analyzed for metals.

BWSs were collected for metals analyses at each of the aforementioned coupon sampling times, with an additional BWS sample collected at 15 minutes after the initiation of the flushing action. Similar to Step 3, the BWSs were preserved in Nalgene™ bottles with 2 drops of concentrated nitric acid.

After the completion of the flushing protocol, the DSS was drained and filled with tap water and fitted with new coupon materials. This initiates the cultivation of biofilm (Step 1) for the next study.

A summary of the 4-stage protocol is presented in Table 1:

Table 1: Pilot Scale Radiological Surrogate Test Protocol

Experimental Phase	PVC Loop Condition	Duration	Loop Flow (gpm)	Fresh Water (gpm)	Sample No.	Sample Type*
Cultivation of Biofilm	Tap water-biofilm growth	7-28 days (minimum)	88	0.8 (addition)	1	DI
					1	CL
Contamination Phase	Radiological surrogate addition to tap water and mix	2 hours	10	0	2	BWS
					1	DI
					1	CL
Decontamination Phase	Drain and fill twice. Decontaminant addition to tap water and mix	20 min	10	0	1	BWS

Experimental Phase	PVC Loop Condition	Duration	Loop Flow (gpm)	Fresh Water (gpm)	Sample No.	Sample Type*
	Static	22 hours	0	0	10	BWS
					9	DI
					9	CL
Flushing	Drain and fill twice. Begin flushing/recirculation with a portion of the water to discharge	22 hours	88	10 (discharge)	4	BWS
					3+1 duplicate	DI
					3+1 duplicate	CL

DI = Ductile iron, CL = Cement lined, BWS = Bulk Water Sample

2.4 Sampling Protocols

2.4.1 Bulk Water Sample Collection

The BWSs were collected using a grab sampling technique into 125 ml Nalgene™ sample bottles that contain two drops of nitric acid to preserve the sample. The sampling port was opened and flushed prior to collection of the samples. Table 2 summarizes the BWS storage and preservation procedures.

Table 2: Bulk Water Sample Storage and Preservation Procedures

Measurement	Sample Container/ Quantity of Sample	Preservation/Storage	Holding Times
Cesium	Nalgene plastic or glass/ 125mL	pH <2 with HNO_3. Refrigerate between 4 ± 2 ºC	6 months
Cobalt	Nalgene plastic or glass/ 125mL	pH <2 with HNO_3. Refrigerate between 4 ± 2 ºC	6 months
Strontium	Nalgene plastic or glass/ 125mL	pH <2 with HNO_3. Refrigerate between 4 ± 2 ºC	6 months

2.4.2 Coupon Sample Collection

The individual coupon sample collection process was described in the experimental protocol section of this report. Once collected, the coupon surface was scraped using a scalpel. The debris scraped from the surface was put directly into a sample bottle containing DI water. Scraping of the iron coupons resulted in all corrosion material being removed, so that only bare, un-oxidized iron was present. Cement mortar coupons were scraped until the substratum was visible. The coupon surface and the scalpel were then rinsed using the least amount of DI water possible. The rinsate was also put directly into the sample bottle. Sample bottles are labeled and refrigerated.

The collection of biofilm for HPC and metals analysis from the coupons is further described in CB&I T&E SOP [Standard Operating Procedure] 210: Biofilm Sample Collection from Coupons

in the Distribution System Simulator (DSS) Pipe-Loop System (Appendices A and B, respectively).

2.4.3 Analytical Methods

After the completion of the specific experiment, the samples were prepared and analyzed in accordance to the methods identified in Table 3.

Table 3: Sample Preparation and Analytical Method Summary

Measurement	Sample Preparation Method	Analysis Method
HPC	CB&I T&E SOP 210 (Appendix A)	CB&I T&E SOP 304 (Appendix B)
Cesium	BWS Samples: USEPA Method 3015A; Coupon Samples: USEPA Method 3051A	USEPA Method 200.9 (GFAA)
Cobalt	BWS Samples: USEPA Method 3015A; Coupon Samples: USEPA Method 3051A	USEPA Method 6010C/ USEPA Method 200.7 (ICP OES)
Strontium	BWS Samples: USEPA Method 3015A; Coupon Samples: USEPA Method 3051A	USEPA Method 6010C/ USEPA Method 200.7 (ICP OES)

HPC, heterotrophic plate count; ICP, inductively coupled plasma; OES, optical emission spectra; GFAA, graphite furnace atomic adsorption

Sources: USEPA Method 200.7, Revision 5 (2001): Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry.

USEPA Method 200.9, Revision 2.2 (1994): Determination of Trace Elements by Stabilized Temperature Graphic Furnace Atomic Absorption.

USEPA Method 3015A (SW846), Revision 1 (2007): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts.

USEPA Method 3051A (SW846), Revision 1 (2007): Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils.

USEPA Method 6010C (SW846), Revision 3 (2000): Inductively Coupled Plasma - Atomic Emission Spectrometry.

2.5 Quality Assurance/Quality Control

All methods and protocol described throughout Section 2.0 were performed in accordance with the EPA Quality Assurance (QA) program. The methods used to analyze HPC, cesium, strontium and cobalt are referenced in Table 3. The controls, QA/Quality Control (QC) criteria, frequency of the control and acceptance criteria are listed in Table 4. No significant deviations from the QAPP were encountered, although two observations should be noted. First in the Results section (Section 3), it is noted in Tables 5 and 6 that some samples were lost during processing, and no data are reported for those samples. Second, it should also be noted that in experiments with cobalt and strontium, concentrations less than the target concentration were detected in the bulk phase immediately after contaminant injection. This was attributed to sorption of the contaminants to the DSS pipe wall soon after injection, which is supported by the data in Tables 6 and 7. However, due to the adsorption, the initial target concentrations could not be achieved.

Table 4: QA/QC criteria and controls for HPC, cesium, cobalt and strontium analyses

Measurement	QA/QC Check	Frequency	Acceptance Criteria	Corrective Action
HPC	Negative Control	Before every set of measurements	No fluorescent wells	Re-analyze sterile buffer and change it if necessary.
HPC	Positive Control	Once per experiment	Fluorescent wells	Investigate laboratory technique. Re-analyze.
HPC	Duplicate	Once per experiment	Duplicate plates much agree within 5%	Investigate laboratory technique. Re-analyze.
Cesium	Calibration	Daily	R2 > 0.998	Investigate issues. Prepare new standards if necessary and recalibrate
Cesium	Quality Control Sample (QCS) (Second Source, external to the laboratory)	After Calibration	±10%	Reanalyze. If fails: Prepare again and reanalyze. If fails: Prepare new calibration standards and recalibrate
Cesium	Instrument Performance Check (IPC) Calibration Verification	After Calibration, every 10 samples and at the end of the analysis batch	±10%	Reanalyze. If fails: Prepare again and reanalyze. If fails: Reanalyze samples not bracketed by appropriate QC
Cesium	Laboratory Reagent Blank (LRB)	One per batch	Analyte Concentration must be less than 10% of the lowest Calibration Standard	Investigate source of contamination and reanalyze
Cesium	Laboratory Fortified Blank (LFB)	One per batch	±15% Spiked Recovery	Investigate source of problem (interference) and reanalyze
Cesium	Laboratory Fortified Matrix (LFM)	One per 10 samples	±30% Spiked Recovery	Investigate source of problem (interference) and reanalyze
Metals (Cobalt and Strontium)	Calibration	Daily	R2 > 0.998	Investigate issues. Prepare new standards if necessary and recalibrate
Metals (Cobalt and Strontium)	Initial Calibration Verification (ICV) (This is a mid-range 2nd source standard)	After Calibration	±10%	Reanalyze. If fails: Prepare again and reanalyze. If fails: Prepare new standards and recalibrate
Metals (Cobalt and Strontium)	Calibration Blank (CB)	After Calibration, every 10 samples and end of analysis batch	Analyte Concentration must be less than 10% of the lowest Calibration Standard	Reanalyze. If fails: Prepare again and reanalyze. If fails: Reanalyze samples not bracketed by appropriate QC

Measurement	QA/QC Check	Frequency	Acceptance Criteria	Corrective Action
Metals (Cobalt and Strontium)	Low Level Calibration Verification (LLCV)	After Calibration and at the end of the analysis batch	±30%	Reanalyze. If fails: Prepare again and reanalyze. If fails: Reanalyze samples not bracketed by appropriate QC
Metals (Cobalt and Strontium)	Continuing Calibration Verification (CCV)	After every 10 samples and end of analysis batch	±10%	Reanalyze. If fails: Prepare again and reanalyze. If fails: Reanalyze samples not bracketed by appropriate QC
Metals (Cobalt and Strontium)	Method Blank	One per sample batch	Analyte Concentration must be less than 10% of the lowest Calibration Standard or lowest Sample Concentration	Reanalyze. If fails: Prepare again and reanalyze. If fails: Prepare entire sample batch again and reanalyze.
Metals (Cobalt and Strontium)	Laboratory Control Sample (LCS)	One per sample batch	±20% Spiked value	Reanalyze. If fails: Prepare again and reanalyze. If fails: Prepare entire sample batch again and reanalyze
Metals (Cobalt and Strontium)	Matrix spike, unspiked duplicate and/or matrix spike duplicate (MS/Dup or MS/MSD)	One per sample batch	20% RSD for duplicates ±25% Spike value recovery	Investigate for interferences or error. Reanalyze.

3.0 Results

3.1 Heterotrophic Plate Count

HPC values on the cement-mortar coupons ranged from 200 to 1350 most probable number (MPN)/in^2 (31 to 209 MPN/cm^2), with an average of 650 MPN/in^2 (101 MPN/cm^2). HPC values on the ductile iron coupons ranged from 800 to 41,000 MPN/in^2 (124 to 6357 MPN/cm^2), with an average of 11,600 MPN/in^2 (1799 MPN/cm^2). The primary reason for measuring HPC on the coupons was to establish that microorganisms had colonized the coupons after one month of conditioning. HPC was generally higher on the ductile iron coupons, which was not surprising since the surface is visibly rougher than the cement-mortar surface. More roughness increases the

surface area with deep recesses that experience less shear from the flow, and decreased or no disinfectant residual due to the iron surface consuming it.

3.2 Cesium Persistence and Decontamination

Table 5 shows the results from duplicate contamination/decontamination experiments with cesium in the DSS. Cesium concentration in the bulk water phase and the amount adhered to corroded iron and cement-mortar infrastructure coupons are displayed. Cesium adhered to corroded iron, but to a lesser degree than cement-mortar. This is consistent with previously reported results showing that cesium reversibly adsorbs to iron oxides, but the adsorption is weak and cesium will desorb in presence of clean water (Ebner et al., 1994; Todorovic et al., 2001). In both experiments, cesium was undetectable on the coupons after application of potassium chloride and flushing. The duplicate experiment had an initial cesium concentration of 21.2 mg/L compared to 13.4 mg/L in the first experiment. However, this difference in initial concentration did not appear to influence the persistence of cesium on either coupon type.

Potassium chloride is a competing ion that will replace cesium adhered to the coupon surface. Since potassium chloride was applied soon after contamination, it is difficult to determine if the cesium removal is due to fresh water being pumped into the DSS pipe between the contamination and decontamination phase, or due to the presence of potassium chloride. However, in both experiments, cesium was detected on the iron coupons after decontamination began, but before becoming undetectable, which suggests that the potassium chloride played a role. In this experiment, it was difficult to separate the effect of flushing alone from that with the potassium chloride.

Table 5: Cesium Contamination and Decontamination Duplicate Results

		12.7 mg/L Cesium chloride (10.0 mg/L Cesium) decontaminated with 0.001 M KCl (74.6 mg/L)			12.7 mg/L Cesium Chloride (10.0 mg/L Cesium) decontaminated with 0.001 M KCl (74.6 mg/L)		
Experimental Activity	Elapsed Time (hrs)	Bulk water Cs concentration (mg/L)	Ductile iron coupon Cs concentration (mg/kg)	Cement lined coupon Cs concentration (mg/kg)	Bulk water Cs concentration (mg/L)	Ductile iron coupon Cs concentration (mg/kg)	Cement lined coupon Cs concentration (mg/kg)
Control	0:00	ND	---	---	ND	---	---
Contaminant Injection	00:00						
	0:05	13.4	---	---	21.2	---	---
	2:00	12.9	3.16	26.7	22.9	2.38	75.9
Decontaminant Phase	0:00						
	0:15	0.574	---	---	0.612	---	---
	0:30	0.434	ND	152.5	0.549	1.44	158.4
	1:00	0.54	1.58	137.1	0.503	1.22	119.8
	1:30	0.125	ND	80.6	0.763	ND	174.2
	2:00	0.102	ND	ND	0.581	ND	90.5
	3:00	0.154	ND	ND	0.579	NA [a]	210.5
	4:00	0.0600	ND	ND	0.637	ND	ND
	6:00	0.228	ND	ND	0.648	ND	72.4
	20:00	0.227	ND	38.3	0.412	ND	ND
	22:00	0.247	ND	ND	0.311	ND	ND
Flushing Phase	0:00						
	0:15	ND	---	---	ND	---	---
	2:00	ND	ND	31.6	ND	ND	ND
	2:00 (dup)	---	ND	9.7	---	ND	ND
	4:00	ND	ND	34.9	ND	ND	39.6
	22:00	ND	ND	21.5	ND	ND	ND

NA[a]:Sample lost during processing; ND: Not Detected; ---: sample not taken

Cesium adherence to cement-mortar was higher than on iron oxides, which is consistent with the literature showing that cesium does adsorb to cement-mortar matrices (Apak et al., 1996). In both experiments with cement mortar coupons, a trend is present where the amount of cesium present on the coupon was higher early in the decontamination phase compared to the end of the contamination phase. This suggests that adherence of the cesium to the cement mortar continued after the last contaminant injection sample was removed (elapsed time of 2 hours). This

phenomenon is also supported by studies showing that cesium adsorption to clays is slow and not instantaneous (Atkinson and Nickerson, 1988; Chorover et al., 2003). Application of potassium chloride did result in removal of cesium, but cesium removal was not consistent over time. Cesium decreased to undetectable levels on some coupons after decontamination started, but was still detected on other coupons. Cesium was also detectable on coupons after flushing began. Some of this may be due to cesium trapped in dead end spaces being remobilized during flushing and subsequently re-adhering to the coupon surfaces.

Two previous studies directly focused on cesium adherence to drinking water infrastructure (Szabo et al., 2009; USEPA, 2014). In Szabo et al., 2009, cesium was not detected on corroded iron coupons in bench scale biofilm anular reactors, after spiking and subsequent flushing with clean water. These results are consistent with the results in this study in the sense that strong cesium adsorption to corroded iron (containing iron oxides) was not observed. The bench scale results suggest that flushing alone would remove cesium from iron coupons, while pilot scale results from this study suggest that addition of potassium chloride enhanced decontamination. In practice, clean water flushing may be effective at removing adhered cesium, but addition of potassium chloride could enhance the decontaminating effect of clean water. It should also be noted that decontamination with 0.001 M KCl required that 0.15 lb (67 g) be dissolved in the pilot scale pipe loop. If this volume were extrapolated to a 400 ft (122 m), 6 inch (15.2 cm) water main between two fire hydrants, 0.4 lb (165.8 g) would be necessary. This mass is easy to handle, and KCl is highly soluble, so this decontamination technique should be implementable in the field.

In USEPA, 2014, cesium initially adhered to cement-mortar coupons when spiked into biofilm annular reactors, but persistence was not observed. Furthermore, simulated flushing in the annual reactors at 1.6 to 2.5 ft/sec (0.50 to 0.75 m/sec) was effective at removing adhered cesium to undetectable levels. Application of potassium chloride and then flushing was effective for some coupons in the pilot scale system, but not others. This result may reveal one of the challenges associated with decontamination on a large scale. The coupons in biofilm annular reactors are consistent in their content and surface smoothness. The coupons in the pilot scale DSS are also consistent in their sand and cement content, but their surface roughness and shape can vary as they are handmade. Cement mortar coatings on pipes in distribution systems should be consistent in their sand and cement content, but different pipes can have varying surface characteristics due to their age or the way they were manufactured. Varying surface characteristics (including biofilm presence) can influence how a contaminant adheres or how a decontaminant interacts with the surface. The inconsistent results observed in the pilot scale DSS may be reflective of the challenges associated with decontaminating real world drinking water infrastructure surfaces.

3.3 Cobalt Persistence and Decontamination

Table 6 shows the results from duplicate contamination/decontamination experiments with cobalt in the DSS.

Table 6: Cobalt Contamination and Decontamination Duplicate Results

		10 mg/L Cobalt (II) chloride (4.48 mg/L Cobalt) decontaminated with 0.01 M EDTA (2,922 mg/L)			10 mg/L Cobalt (II) chloride (4.48 mg/L Cobalt) decontaminated with 0.01 M EDTA (2,922 mg/L)		
Experimental Activity	Elapsed Time (hrs)	Bulk water Co concentration (mg/L)	Ductile iron coupon Co concentration (mg/kg)	Cement lined coupon Co concentration (mg/kg)	Bulk water Co concentration (mg/L)	Ductile iron coupon Co concentration (mg/kg)	Cement lined coupon Co concentration (mg/kg)
Control	00:00	ND	---	---	ND	---	---
Contaminant Injection	00:00						
	0:05	3.553	---	---	3.944	---	---
	2:00	3.057	80.17	52.74	3.618	69.59	131.09
Decontaminant Phase	0:00						
	0:15	0.753	---	---	0.298	---	---
	0:30	0.756	5.18	10.3	0.291	ND	16.48
	1:00	0.751	12.40	7.91	0.280	ND	NA [a]
	1:30	0.751	7.73	7.19	0.284	ND	ND
	2:00	0.747	15.37	8.08	0.276	ND	ND
	3:00	0.747	13.27	7.24	0.273	ND	ND
	4:00	0.746	13.38	6.58	0.282	ND	ND
	6:00	0.750	12.23	5.84	0.280	ND	ND
	20:00	0.731	14.34	3.94	0.282	ND	ND
	22:00	0.736	14.86	ND	0.282	ND	ND
Flushing Phase	0:00						
	0:15	ND	---	---	ND	---	---
	2:00	ND	22.48	ND	ND	ND	ND
	2:00 (dup)	---	7.28	4.14	---	ND	ND
	4:00	ND	6.65	ND	ND	ND	ND
	22:00	ND	25.82	3.76	ND	ND	ND

NA[a]:Sample lost during processing; ND: Not Detected; ---: sample not taken

Cobalt adhered to both corroded iron and cement-mortar drinking water infrastructure coupons. In past studies, it has been observed that when soluble cobalt chloride is introduced into chlorinated water, the soluble cobalt(II) oxidizes to insoluble cobalt(III), and this insoluble material precipitates on and sticks to drinking water infrastructure surfaces (Szabo et al., 2009; USEPA,

2014). Precipitation and adherence of the cobalt was also observed in the pilot scale study. For both corroded iron and cement mortar, EDTA was an effective decontaminating agent. In one of the experiments, cobalt was reduced to non-detectable levels (>99.99% removal), while in the other 80% to 93% cobalt decontamination was observed. In the experiments where EDTA did not reduce cobalt to undetectable levels, flushing after application of EDTA did not result further removal of cobalt.

The discrepancy between the two replicate experiments can be explained by the inability to completely dissolve EDTA in solution. EDTA can be dissolved up to 0.26 M (Appendix C), and was introduced into the DSS at 0.01 M. However, EDTA dissolves slowly, and it can be influenced by pH. EDTA is more soluble at pH of 4 to 6 or above. The pH of the Cincinnati tap water used in the DSS ranged from 8.0 to 8.5, and it was expected that it would have enough buffering capacity to keep the pH in the 4.0 to 6.0 range after introduction of EDTA. However, after introduction of EDTA, pH dropped to between 3.0 and 3.5 and remained in that range for the duration of the decontamination experiment. As a result, precipitated EDTA was observed sitting at the bottom of the DSS pipe. Some EDTA was dissolved, but the dissolved amount in contact with the coupons was likely different in each experiment.

In Szabo et al., 2009, precipitated cobalt was extracted from iron coupons in bench scale biofilm annular reactors with 0.36 M sulfuric acid, which removed over 90% of the adhered cobalt. In the pilot scale studies, 0.01 M EDTA resulted in 80 to 93% cobalt removal in one experiment and >99.99% (non-detectable level of cobalt) in a second identical study. EDTA at 0.01 M compares favorably with acid treatment. Although pH dropped to a range between 3.0 and 3.5 during EDTA treatment, this pH level is preferable than the intensely corrosive conditions resulting from sulfuric acid treatment, which removed cobalt by dissolving the iron surface. However, achieving 0.01 M EDTA in the pipe loop required 5.8 lbs (2,655 g) of EDTA. If this mass were extended to a 400 ft (122 m), 6 inch (15.2 cm) water distribution pipe between two fire hydrants, 14 lbs of EDTA would be required.

The results in USEPA, 2014 showed that cobalt was persistent on cement-mortar coupons in bench scale biofilm annular reactors. Flushing at 1.6 to 2.5 ft/sec (0.50 to 0.75 m/sec) had no effect on the adhered cobalt. Treatment with 0.1 M EDTA removed 95% of the cobalt adhered to cement mortar. There was no mention of whether EDTA precipitation was observed in USEPA, 2014. The results of the pilot scale study reported here compare favorably to those of USEPA, 2014. This is especially noteworthy given that a lower concentration of EDTA was used in the pilot studies. Together, the bench and pilot scale results indicate that EDTA is a good decontamination agent for cobalt adhered to cement mortar. However, when implementing EDTA decontamination in a real distribution system, care will have to be taken to ensure that full dissolution of the EDTA

occurs. Co-injection of a basic compound like sodium hydroxide could help keep the pH in the 4.0 to 6.0 range (or higher), which could facilitate EDTA dissolution.

3.4 Strontium Persistence and Decontamination

Table 7 shows the results from duplicate contamination/decontamination experiments with strontium in the DSS. Like cesium, strontium adhered more to cement-mortar than corroded iron. Strontium was detected on both materials after decontamination with 0.01 M ammonium acetate and subsequent flushing. For ductile iron, decontamination effectiveness in the two experiments was 23% to 31% in the 30 minutes after ammonium acetate was applied, and up to 50% to 87% by the end of the treatment phase (22 hrs). For cement-mortar, decontamination ranged from 13% to 28% in the first experiment and 11% to 94% in the second experiment. Like in the cesium experiments, the variability of strontium concentration on the coupons during decontamination is attributed to variability in the surface characteristics such as roughness and the presence of biofilm. It is presumed that in reality, the same type of pipe material can vary in surface roughness and composition of cement/sand or impurities in iron due to different pipe ages and manufacturers.

Strontium adsorption to iron oxides, which make up most of the iron corrosion matrix on water pipe interiors, has been studied in small, bench scale experiments. In general, these bench scale studies have found that strontium adsorption is transient and reversible, particularly if iron oxides are exposed to clean water after application of strontium (Axe et al., 1998; Gerke et al., 2014; Small et al., 1999). However, strontium is far more persistent on calcium carbonate (calcite), which can deposit on water infrastructure (Carroll et al., 2008). Strontium and calcium are neighbors in the alkaline earth metals column of the periodic table and share many similar properties. Strontium exchange with calcium carbonate compounds may also promote strontium adhesion on goethite and other iron oxides. These phenomena may explain the persistence of strontium on the corroded iron coupon and the inability of ammonium acetate to completely remove it.

USEPA, 2014 contains persistence and decontamination results for strontium on cement-mortar water infrastructure. Like the results of this pilot scale study, the results in USEPA, 2014 in ARs show that strontium readily adheres to cement mortar infrastructure. The results also show that strontium is persistent on cement-mortar, but flushing at 1.6 to 2.5 ft/sec (0.50 to 0.75 m/sec) removes approximately 40% of the adhered strontium. Application of ammonium acetate at 0.2 M removed 90% of the adhered strontium. The results of this study support those generated from bench scale biofilm annular reactor studies. More strontium was removed from cement-mortar in the bench scale study, but 20 times more ammonium acetate was applied on the bench scale compared to the pilot scale experiments.

The difference in results between the bench scale and pilot scale studies highlight one of the challenges of translating bench scale data to a pilot scale setup or real distribution system. Ammonium acetate was an effective decontamination agent when applied at 0.2 M in bench scale experiments, but less so when applied at 0.01 M (20-fold less) in the pilot scale experiments. Achieving 0.01 M ammonium acetate in the pilot scale pipe loop required dissolving 1.5 lbs (681 g) of the chemical in the entire volume. A concentration of 0.2 M would require 30 lbs (13,620 g), which was deemed too much for this experiment. For comparison, consider a 400 ft (122 m), 6 inch (15.2 cm) diameter pipe between two fire hydrants. Decontaminating this volume with ammonium acetate at 0.2 M would require 75 lb (34,050 g), while 0.01 M would require 3.7 lb (1,680 g). Therefore, ammonium acetate may be a good decontamination method for strontium adhered to cement-mortar, but the amount that would need to be added to a real distribution system would be large enough that its application may be prohibitive over a large area. Adequate decontamination of adhered strontium may require a physical removal operation (e.g., pigging) in addition to flushing and ammonium acetate injection.

Table 7: Strontium Contamination and Decontamination Duplicate Results

		10 mg/L Strontium chloride (5.46 mg/L Strontium) decontaminated with 0.01 M Ammonium acetate (770.8 mg/L)			10 mg/L Strontium chloride (5.46 mg/L Strontium) decontaminated with 0.01 M Ammonium acetate (770.8 mg/L)		
Experimental Activity	Elapsed Time (hrs)	Bulk water Sr concentration (mg/L)	Ductile iron coupon Sr concentration (mg/kg)	Cement lined coupon Sr concentration (mg/kg)	Bulk water Sr concentration (mg/L)	Ductile iron coupon Sr concentration (mg/kg)	Cement lined coupon Sr concentration (mg/kg)
Control	00:00	0.172	---	---	0.318	---	---
Contaminant Injection	00:00						
	0:05	3.900	---	---	5.924	---	---
	2:00	3.732	69.99	1,459.0	5.381	96.80	2,433.4
Decontaminant Phase	0:00						
	0:15	0.330	---	---	0.317	---	---
	0:30	0.342	48.23	1,077.5	0.274	56.57	1,470.7
	1:00	0.332	46.41	1,107.3	0.315	74.77	262.7
	1:30	0.328	43.49	1,045.0	0.322	51.87	2,152.6
	2:00	0.326	41.10	1,103.7	0.333	46.29	239.7
	3:00	0.334	40.65	1,274.4	0.315	12.59	1,386.7
	4:00	0.344	43.94	1,318.2	0.387	66.59	195.8
	6:00	0.334	41.81	1,276.3	0.274	45.64	1,264.6
	20:00	0.342	38.00	1,269.6	0.258	24.01	862.9
	22:00	0.336	34.68	1,553.2	0.267	28.15	125.1
Flushing Phase	0:00						
	0:15	0.170	---	---	0.167	---	---
	2:00	0.166	36.73	1,340.4	0.168	32.14	119.7
	2:00 (dup)	---	41.16	1,008.1	---	33.94	116.6
	4:00	0.166	37.89	1,202.5	0.157	9.81	443.2
	22:00	0.162	44.62	996.6	0.172	34.52	2,046.2

4.0 Conclusions

This study produced pilot-scale decontamination data for non-radioactive cesium, cobalt and strontium adhered to corroded iron and cement-mortar drinking water infrastructure. This pilot-scale data was also compared to decontamination data generated on the bench scale under similar conditions. The key findings are as follows:

- Cesium was not persistent on corroded iron in the presence of 0.001 M potassium chloride on the pilot scale. The bench scale results suggest that flushing alone would remove cesium from corroded iron. In practice, clean water flushing may be effective at removing adhered cesium from iron, but addition of potassium chloride could enhance the decontamination effect of clean water. Cesium was more persistent on cement-mortar relative to iron, but 0.001 M potassium chloride was an effective decontaminant. Bench scale data showed that flushing with clean water removed cesium from cement-mortar coupons. If decontamination of cesium from water infrastructure in the field is necessary, the data suggests that flushing and application of potassium chloride are effective decontamination methods. It should be noted that the pilot scale results showed that not all coupons were decontaminated equally, which may also hold could true in a real water distribution system with pipe materials of different ages and from different manufacturers.
- Cobalt adhered to corroded iron and cement-mortar water infrastructure, and 0.01 M EDTA was an effective decontaminant for both surfaces. These results are consistent with decontamination data generated on the bench scale. However, in the pilot scale experiments, it was noticed that EDTA did not fully dissolve in the pipe loop. This may have affected the concentration of EDTA in the pipe, which resulted in inconsistent level of decontamination between experiments. The lack of full EDTA dissolution was attributed to a drop in pH, which negatively affected EDTA solubility. When implementing EDTA decontamination in a real distribution system, care will have to be taken to ensure that full dissolution of the EDTA occurs. Co-injection of a basic compound like sodium hydroxide could help keep the pH in the 4.0 to 6.0 range (or higher), which could facilitate EDTA dissolution and make it more potent.
- Strontium adhered to both corroded iron and cement-mortar, but the amount of initial persistence on cement-mortar was 20 to 25 time higher than iron. Ammonium acetate (0.01 M) did remove the adhered strontium, but not to the extent observed in bench scale studies using 0.2 M. Decontaminating the pilot scale pipe loop required dissolving 1.5 lbs (681 g) of the chemical in the entire volume. A concentration of 0.2 M would have require 30 lbs (13,620 g). Extrapolated to a 400 ft (122 m), 6 inch (15.2 cm) diameter water pipe, 0.2 M would require 75 lb (34,050 g), while 0.01 M would require 3.7 lb (1,680 g) of ammonium acetate. Due to the large amount of ammonium acetate needed to achieve 0.2 M in a real water distribution system, adequate decontamination of adhered strontium may require a

physical removal operation (e.g., pigging) in addition to flushing and ammonium acetate injection.

5.0 References

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EPA T&E Contract Technical Standard Operating Procedure

BIOFILM SAMPLE COLLECTION FROM COUPONS IN THE DISTRIBUTION SYSTEM SIMULATOR (DSS) PIPE-LOOP SYSTEM

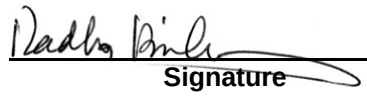
T&E SOP 210

Revision Number: 0

Date: 11/24/2014

SOP Approval

1. E. Radha Krishnan, P.E.
Program Manager



Signature **11/24/2014**
Date

2. Steven Jones, ASQ CQA/CQE
Quality Assurance Manager

Signature **11/24/2014**
Date

Revision Summary

Revision	Name	Date	Description of Change
0	Nicole Sojda/ Lee Heckman	11/24/2006	Developed SOP.

TABLE OF CONTENTS

1.0 SCOPE AND APPLICABILITY

1.1 The method described in this standard operating procedure (SOP) is applicable to the collection of biofilm samples from coupons in the Distribution System Simulator (DSS) pipe-loop system for purposes of determining the viability of biofilm growth in the DSS and determining the effectiveness of various decontamination methods on different interior pipe surfaces.

1.2 Coupon samples are fabricated from different pipe materials, such as ductile iron, concrete, and PVC. Coupon fabrication and installation in the DSS are discussed in T&E SOP 208, *Operation of the Distribution System Simulator (DSS) Pipe-loop System*.

2.0 SUMMARY OF METHOD

Coupons of different pipe materials are inserted into the DSS, and naturally occurring biofilm is allowed to accumulate for approximately thirty (30) days. Water containing contaminants of interest are introduced into the DSS and recirculated, allowing them an opportunity to adhere to the coupons. The DSS is then decontaminated. Coupons of the different pipe materials are removed from the DSS at various times to evaluate the effectiveness of the decontamination process. The surface of each coupon is scraped using a sterilized surgical scalpel to collect biofilm sample for microbiological analysis. Extracted materials are collected in 100mL coliform sample vials with a sodium thiosulfate tablet, and 90mL of sterile phosphate buffer. Large pieces of coupon material are ground using a sterile metal rod. Coupon samples can be pasteurized immediately, or held at 4°C for later pasteurization.

3.0 DEFINITIONS

3.1 Biofilm – An aggregation of microorganisms that forms a thin coating on a substrate.

3.2 Coupon – A replaceable plug that is used as the substrate upon which biofilm is grown.

4.0 HEALTH AND SAFETY WARNINGS

4.1 Standard laboratory personal protective equipment (PPE) (i.e., lab coat, gloves, safety glasses, and steel toed boots) is required.

4.2 The biohazards and the risk of infection by pathogens associated with this method are minimal.

4.3 Exposure to ultra-violet (UV) radiation is minimal. An interlock device in the Millipore UV sterilizer switches off the UV lamps when the lid is opened.

5.0 CAUTIONS

5.1 Avoid touching coupon surfaces prior to scraping to prevent biofilm loss.

5.2 Ensure scalpels and metal rods are exposed to UV light at least two minutes before use. This will prevent cross-contamination from prior uses.

5.3 Do not use the scalpel/metal rod pair designated for ductile iron coupons on cement-lined coupons, and vice versa. There are dedicated scalpels and metal rods for each coupon type. For example, scalpels marked “I” for ductile iron should only be used for ductile iron coupons, and scalpels marked “C” should only be used for concrete coupons.

5.4 To prevent spilling sample, gently grind large pieces of coupon material with the appropriate metal rod.

6.0 INTERFERENCES

None anticipated.

7.0 PERSONNEL QUALIFICATIONS

The techniques of a first-time analyst must be reviewed by an experienced analyst prior to initiating this SOP alone. During this review, the new analysts will be expected to demonstrate their capability to perform this procedure.

8.0 EQUIPMENT AND SUPPLIES

8.1 100mL coliform sample bottles with sodium thiosulfate tablet.

8.2 Scalpels.

8.3 Metal rods.

8.4 Millipore UV sterilizer.

8.5 Petri plates.

9.0 MEDIA AND REAGENTS

9.1 Dilution blanks containing 90 mL phosphate buffer with magnesium chloride.

9.2 Deionized water in squeeze bottle.

10.0 PROCEDURE

10.1 Sample Collection, Handling, and Preservation

10.1.1 After removing coupons from DSS, place each in one half of a petri plate for transport to the BSL-2 Laboratory.

10.1.2 Use one 100 mL coliform sample bottle per sample. Pour approximately half of a 90 mL dilution blank into the coliform sample bottle. The remaining portion of the dilution blank may be used for a second sample.

10.1.3 Remove the scalpel from the UV sterilizer designated for the coupon type to be scraped. Close the UV sterilizer.

10.1.4 If the surface of the coupon appears dry, wet the surface with deionized water from the squeeze bottle while holding coupon above the open coliform sample bottle to collect any contact water runoff.

10.1.5 Holding the coupon over the open pre-labeled coliform sample bottle, gently scrape the coupon surface with a scalpel into the 100 mL coliform sample bottle.

- Ductile iron coupons should have a coating of iron oxide. This may fall off in pieces, or in a single piece when scraped.
  - Rinse the iron coupon surface with deionized water, and scrape the exposed iron. Rinse with deionized water.
- Cement-lined coupons may have cement on the periphery of the coupon. This is to be collected by scraping it into the buffer.

- Scrape the surface of the coupon's cement plug. A slight change in color may be observed. Rinse with deionized water.

- Rinse the scalpel with deionized water into the 100 mL coliform sample bottle.

10.1.6 Place the scalpel back in the UV sterilizer. Remove the metal rod designated for grinding extracted coupon material of the coupon type. The large diameter rod is to be used for ductile iron. The small diameter rod is to be used for cement-lined coupons.

10.1.7 Close the UV sterilizer. Grind the extracted coupon material so that no splashing occurs.

10.1.8 Rinse the metal grinding rod with deionized water into the 100 mL coliform sample bottle.

10.1.9 Place the metal grinding rod into the UV sterilizer and close the lid.

10.1.10 Fill the coliform sample bottle with deionized water to the 100 mL fill line.

10.2 Sample Storage

10.2.1 Extracted coupon samples can to be stored at 4°C up to 24 hours. They must then be pasteurized/heat shocked.

10.2.2 Pasteurized/heat shocked samples may be stored at 4°C up to 48 hours before analysis using the spread plate method. Pasteurization/heat shocking is detailed in Section 9.6 of T&E SOP 309, *Preparation and Enumeration of B. globigii Endospores*.

10.3 Analysis

10.3.1 Conduct microbiological sample analysis in accordance with the analysis-specific SOP or method.

11.0 DATA AND RECORDS MANAGEMENT

11.1 All original analytical documentation generated and prepared for the EPA shall be controlled in accordance with T&E SOP 101, *Central Files*.

11.2 All data packages shall be assembled and reviewed per T&E SOP 102, *Data Review and Verification*.

12.0 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 One field duplicate coupon is collected from the DSS at a frequency of one per experiment. Acceptance criteria for the field duplicate is ≤20% variation or as specified in the project-specific Quality Assurance Project Plan (QAPP).

12.2 Analysis-specific QA/QC requirements are specified in the analysis-specific SOP or method.

13.0 REFERENCES

13.1 EPA, March 2001. *Guidance for Preparing Standard Operating Procedures* (EPA QA/G-6), EPA/240/B-01/004, Office of Environmental Information.

13.2 CB&I Federal Services LLC, 2011. EPA T&E Contract Administrative SOP 101, *Central Files*.

13.3 CB&I Federal Services LLC, 2011. EPA T&E Contract Administrative SOP 102, *Data Review and Verification*.

13.4 CB&I Federal Services LLC, 2010. EPA T&E Contract Technical SOP 208, Operation of the Distribution System Simulator (DSS) Pipe-loop System.

13.5 CB&I Federal Services LLC, 2012. EPA T&E Contract Technical SOP 309, *Preparation and Enumeration of B. globigii Endospores*.

Shaw™
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EPA T&E Contract Technical Standard Operating Procedure

Heterotrophic Plate Count (HPC) Analysis Using IDEXX SimPlate Method

T&E SOP 304

Revision Number: 1
Revision Date: 02/08/2012

SOP Approval

1. E. Radha Krishnan, P.E.
Program Manager

Signature — Date: 02/10/2012

2. Steven Jones, ASQ CQA/CQE
Quality Assurance Manager

Signature — Date: 02/10/2012

Revision Summary

Revision	Name	Date	Description of Change
0	Nur Muhammad	01/31/2006	Developed SOP.
1	Nancy Shaw/ Steven Jones	01/25/2012	Revised Sections 1, 2, 4, 6, 7, 8, 9.2, 10 and 12. Added Attachments A and B.

TABLE OF CONTENTS

1.0 Scope and Applicability

The method described in this standard operating procedure (SOP) is applicable to the enumeration of heterotrophic bacteria, generally known as heterotrophic plate counts (HPC), in water and wastewater samples.

2.0 Summary of Method

IDEXX SimPlate method for quantification of HPC is based on multiple enzyme technology which detects viable bacteria in water by testing for the presence of key enzymes known to be present in these organisms. It uses multiple enzyme substrates that produce a blue fluorescence when metabolized by bacteria. The sample and media are added to a SimPlate plate, incubated and then examined for fluorescing wells. The number of fluorescing wells corresponds to a Most Probable Number (MPN) of total bacteria in the original sample.

3.0 Definitions

3.1 HPC – Heterotrophic Plate Count

3.2 IDEXX – Biological system and reagent developing company.

3.3 SimPlate – Registered trademark of BioControl Systems Inc., and is used by IDEXX under license from BioControl System Inc.

4.0 Health and Safety Warnings

4.1 Standard laboratory personal protective equipment (i.e., laboratory coat, gloves, and safety glasses) is required. In addition, any chemical-specific or project-specific protective gear required will be described in the project-specific Health and Safety Plan (HASP).

4.2 If using an ultraviolet (UV) light system without a viewing chamber, wear UV protective safety glasses and direct light away from eyes.

4.3 Special precautions, such as wearing heat-resistant gloves, are required for autoclaving.

5.0 Cautions

Samples collected for analysis in accordance with this Standard Operating Procedure (SOP) shall be preserved at 4±2 ^{o}C after collection and processed preferably within 48 hours after sample collection.

6.0 Interferences

6.1 Contamination during analysis affects the results. Aseptic technique should be followed during analysis.

6.2 Chlorinated samples should be treated with sodium thiosulfate prior to testing.

7.0 Personnel Qualifications

The techniques of a first time analyst shall be reviewed by an experienced analyst prior to initiating this SOP alone. During this review, the new analysts will be expected to demonstrate their capability to perform this analysis.

8.0 Equipment and Supplies

8.1 IDEXX multi dose sterile media

8.2 IDEXX sterile SimPlate plates with lids

8.3 10 ml sterile disposable pipettes

8.4 Sterile dilution buffer (90 ml vials) from Hardy Diagnostics (www.hardydiagnostics.com; Cat # D690)

8.5 UV light set (6 watt, 365 nm) with viewing chamber

8.6 Incubator capable of maintaining a temperature of 35±0.5 ^{0}C

8.7 SimPlate® For HPC Most Probable Number (MPN) Table (supplied with the IDEXX media and plates)

8.8 100 ml sampling bottles with sodium thiosulfate (0.01% w/v) (Fisher Scientific, Cat..No. 09 730 91)

8.9 Autoclave capable of sterilizing with fast, liquid, and dry cycles

9.0 Procedure

9.1 Sample Collection, Handling, and Analysis

9.1.1 Use 100 ml sampling bottles containing sodium thiosulfate for sample collection.

9.1.2 Samples should be transported to the laboratory immediately and stored at 4±2 ^{0}C until processed.

9.1.3 Samples should be processed within 48 hours of sample collection.

9.2 Media Preparation and Sample Analysis

9.2.1 Open the IDEXX multi dose media vessel and add 100 ml sterile dilution buffer. Re-cap the vessel and shake to dissolve the media properly.

9.2.2 Prepare serial dilutions of the sample if necessary.

9.2.3 Pipette 1 ml sample and then 9 ml of the re-hydrated IDEXX multi dose media onto the center of an IDEXX SimPlate plate base.

9.2.4 Cover the SimPlate plate with lid and gently swirl to distribute the sample into all the wells.

9.2.5 Tip the plate 90 – 120^{0} to drain excess sample into the absorbent pad.

9.2.6 Invert the plate, and incubate for 45 - 72 hours at 35±0.5 ^{0}C.

9.2.7 Remove cover and put the plate in the UV system viewing chamber. Turn the UV light (Section 8.5) on 5 inches above the plate, and count the number of fluorescent wells.

9.2.8 Refer to the SimPlate® For HPC Most Probable Number (MPN) Table (see Attachment A) to determine the MPN of heterotrophic plate count bacteria in the original sample. Report the MPN to reflect the dilution used. For example, if 1 mL of a 1:10 dilution of the original sample was tested, then the reported MPN is the table number multiplied by 10 and the result is reported as MPN per 10 mL.

9.2.9 Record the analysis date, dilutions, number of fluorescence wells and heterotrophic bacterial counts on Attachment B, *Datasheet for Heterotrophic Plate Count Analysis*.

9.2.10 Autoclave the plates to sterilize, and dispose of the plates.

9.2.11 Refrigerate any unused rehydrated media and discard after 5 days if not used.

9.2.12 Store dehydrated media in the dark at room temperature.

10.0 Data and Records Management

10.1 All original analysis documentation generated and prepared for the U.S. Environmental Protection Agency (EPA) shall be controlled in accordance with Shaw T&E SOP 101, *Central Files*.

10.2 All data packages shall be assembled and reviewed per Shaw T&E SOP 102, *Data Review and Verification*.

11.0 Quality Control and Quality Assurance

11.1 Negative Control - test a negative control following the test procedure using 10 ml re-hydrated media before every set of measurements. No wells should fluorescence after incubation. In case of failure, use a new media vessel and dilution buffer.

11.2 Positive Control – test a positive control following the test procedure using 10 mL dechlorinated tap water to rehydrate the media. An acceptable positive control should yield 10 – 30 fluorescent wells (21 – 74 MPN) or more. To dechlorinate, add tap water to 100 ml sampling bottle containing sodium thiosulfate (Section 8.8).

11.3 Duplicate - for verification purposes, perform tests in duplicate per sample dilution and for each positive control. Counts from duplicate plates must agree within 5%.

12.0 References

12.1 IDEXX. *Instructional Manual for SimPlate for HPC Multi Dose*, Maine, USA.

12.2 Shaw Environmental & Infrastructure, Inc., 2011. EPA T&E Contract Administrative SOP 101, *Central Files*.

12.3 Shaw Environmental & Infrastructure, Inc., 2011. EPA T&E Contract Administrative SOP 102, *Data Review and Verification*.

12.4 Standard Methods for the Examination of Water and Wastewater, 20th edition, 1998. Method 9215 A, Heterotrophic Plate Count. American Public Health Association.

Attachment A – MPN Tables (Page 1 of 2)

Unit-Dose
SimPlate® For HPC
Most Probable Number (MPN) Table

# Positive Wells	MPN	95% confidence limits	
		lower	upper
0	<0.2	<0.03	<1.4
1	0.2	0.03	1.4
2	0.4	0.1	1.6
3	0.6	0.2	1.9
4	0.8	0.3	2.2
5	1.0	0.4	2.5
6	1.2	0.6	2.7
7	1.5	0.7	3
8	1.7	0.8	3.3
9	1.9	1	3.6
10	2.1	1.1	3.9
11	2.3	1.3	4.2
12	2.6	1.5	4.5
13	2.8	1.6	4.8
14	3.0	1.8	5.1
15	3.3	2.0	5.4
16	3.5	2.2	5.8
17	3.8	2.3	6.1
18	4.0	2.5	6.4
19	4.3	2.7	6.7
20	4.5	2.9	7
21	4.8	3.1	7.4
22	5.1	3.3	7.7
23	5.3	3.5	8.0
24	5.6	3.8	8.4
25	5.9	4	8.7
26	6.2	4.2	9.1
27	6.5	4.4	9.4
28	6.8	4.7	9.8
29	7.1	4.9	10.2
30	7.4	5.1	10.6
31	7.7	5.4	10.9
32	8.0	5.6	11.3
33	8.3	5.9	11.7
34	8.6	6.2	12.1
35	9.0	6.4	12.6
36	9.3	6.7	13.0
37	9.7	7	13.4
38	10.0	7.3	13.9
39	10.4	7.6	14.3
40	10.8	7.9	14.8
41	11.2	8.2	15.2
42	11.6	8.5	15.7

# Positive Wells	MPN	95% confidence limits	
		lower	upper
43	12.0	8.8	16.2
44	12.4	9.1	16.7
45	12.8	9.5	17.3
46	13.2	9.8	17.8
47	13.7	10.2	18.3
48	14.1	10.6	18.9
49	14.6	10.9	19.5
50	15.1	11.3	20.1
51	15.6	11.7	20.7
52	16.1	12.1	21.3
53	16.6	12.5	22.0
54	17.1	13.0	22.7
55	17.7	13.4	23.4
56	18.3	13.9	24.1
57	18.9	14.4	24.9
58	19.5	14.9	25.7
59	20.2	15.4	26.5
60	20.9	15.9	27.3
61	21.6	16.5	28.2
62	22.3	17.1	29.2
63	23.1	17.7	30.2
64	23.9	18.3	31.2
65	24.8	19.0	32.3
66	25.7	19.7	33.5
67	26.6	20.4	34.7
68	27.6	21.2	36.1
69	28.7	22.0	37.5
70	29.9	22.9	39.0
71	31.1	23.8	40.7
72	32.4	24.8	42.5
73	33.9	25.8	44.4
74	35.5	27.0	46.6
75	37.2	28.2	49.1
76	39.2	29.6	51.9
77	41.4	31.1	55.1
78	44.0	32.8	58.9
79	47.0	34.8	63.6
80	50.7	37.1	69.5
81	55.5	39.8	77.5
82	62.3	43.2	89.9
83	73.8	47.6	114.6
84	>73.8	>47.6	>114.6

MPN is per ml of sample (pour-off is accounted for).

Attachment A – MP^N Tables (Page 2 of 2)

Multi-Dose
SimPlate® For HPC
Most Probable Number (MPN) Table

# Positive Wells	MPN	95% confidence limits	
		lower	upper
0	<2	<0.3	<14
1	2	0.3	14
2	4	1	16
3	6	2	19
4	8	3	22
5	10	4	25
6	12	6	27
7	15	7	30
8	17	8	33
9	19	10	36
10	21	11	39
11	23	13	42
12	26	15	45
13	28	16	48
14	30	18	51
15	33	20	54
16	35	22	58
17	38	23	61
18	40	25	64
19	43	27	67
20	45	29	70
21	48	31	74
22	51	33	77
23	53	35	80
24	56	38	84
25	59	40	87
26	62	42	91
27	65	44	94
28	68	47	98
29	71	49	102
30	74	51	106
31	77	54	109
32	80	56	113
33	83	59	117
34	86	62	121
35	90	64	126
36	93	67	130
37	97	70	134
38	100	73	139
39	104	76	143
40	108	79	148
41	112	82	152
42	116	85	157

# Positive Wells	MPN	95% confidence limits	
		lower	upper
43	120	88	162
44	124	91	167
45	128	95	173
46	132	98	178
47	137	102	183
48	141	106	189
49	146	109	195
50	151	113	201
51	156	117	207
52	161	121	213
53	166	125	220
54	171	130	227
55	177	134	234
56	183	139	241
57	189	144	249
58	195	149	257
59	202	154	265
60	209	159	273
61	216	165	282
62	223	171	292
63	231	177	302
64	239	183	312
65	248	190	323
66	257	197	335
67	266	204	347
68	276	212	361
69	287	220	375
70	299	229	390
71	311	238	407
72	324	248	425
73	339	258	444
74	355	270	466
75	372	282	491
76	392	296	519
77	414	311	551
78	440	328	589
79	470	348	636
80	507	371	695
81	555	398	775
82	623	432	899
83	738	476	1146
84	>738	>476	>1146

MPN is per ml of sample (pour-off is accounted for).

Attachment B – Datasheet for Heterotrophic Plate Count Analysis

Analysis Date: _____ Work Assignment: _____

Sterile Dilution Buffer (for negative control) Lot #: _____ Exp. Date: _____

Sodium Thiosulfate Bottle (for positive control) Lot #: _____ Exp. Date: _____

Sample ID	Dilution Factor	# of Fluorescent Wells	Heterotrophic Bacteria (MPN / mL)	Heterotrophic Bacteria x dilution factor (MPN / mL)

Quality Control

Negative control buffer analyzed? ☐ Yes ☐ No

Negative control results acceptable (no yellow or fluorescent wells)? ☐ Yes ☐ No

Positive control results acceptable (5 – 30 fluorescent wells)? ☐ Yes ☐ No

Comments: _____

_____

_____

Analyst: _____ Date: _____

Reviewed by: _____ Date: _____

Appendix C: Safety Data Sheet for EDTA

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ProductInformation

Ethylenediaminetetraacetic acid disodium salt dihydrate

Product Number **E 5134**
Store at Room Temperature

Product Description

Molecular Formula: $C_{10}H_{14}N_2Na_2O_8 \bullet 2H_2O$
Molecular Weight: 372.2
CAS Number: 6381-92-6
Melting Point: 248 °C
pK_a: 2.0, 2.7, 6.2, 10.3[1]

Synonyms: EDTA, (Ethylenedinitrilo)tetraacetic acid

This product is designated as Molecular Biology grade and is suitable for molecular biology applications. It has been analyzed for the presence of nucleases and proteases.

EDTA is an inhibitor of metalloproteases, at effective concentrations of 1-10 µM. EDTA acts as a chelator of the zinc ion in the active site of metalloproteases, and can also inhibit other metal ion-dependent proteases such as calcium-dependent cysteine proteases. EDTA may interfere with biological processes which are metal-dependent.[2]

For use as an anticoagulant, disodium or tripotassium salts of EDTA are most commonly used. The optimal concentration is 1.5 mg per ml of blood. EDTA prevents platelet aggregation and is, therefore, the preferred anticoagulant for platelet counts.[3] Using a 2% EDTA solution, 1-2 drops per ml of whole blood can be used as an anticoagulant.

A procedure for a chromogenic assay of EDTA has been published.[4]

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

This product is slowly soluble in water at room temperature up to 0.26 M, which is approximately 96 mg in a final volume of 1 ml. The pH of this solution will be in the range of 4 to 6. EDTA salts are more soluble in water as the pH increases: the more EDTA there is in the salt form, the higher the pH of a water solution, and therefore, the higher the room temperature solubility. This can be achieved by a gradual addition of concentrated sodium hydroxide solution to the EDTA solution.

Storage/Stability

A stock solution of 0.5 M at pH 8.5 is stable for months at 4 °C. [2]

Solutions of EDTA may be autoclaved.

References

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3. Clinical Hematology: Principles, Procedures, Correlations. ed. Lotspeich-Steininger, C. A., et al., Lippincott (Philadelphia, PA: 1992), p. 18.
4. Sorensen, K., An Easy Microtiter Plate-based Chromogenic Assay for Ethylenediaminetetraacetic Acid and Similar Chelating Agents in Biochemical Samples. Anal. Biochem., **206(1)**, 210-211 (1992).

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