MEMORANDUM

DATE: March 3, 2009
TO: Rob Pedersen, Project Officer, OEA
FROM: Stephanie Harris, Technical Director, Microbiology
Region 10 Laboratory
SUBJECT: Final report for the Micro Drysuit Decon
Project Code: LAB 502R
Account Code: 0809B10P202BD4C
cc: Barry Pepich, Laboratory Director
USEPA Region 10

The following is a final report discussing the results for the Drysuit Decon Study using *Pseudomonas aeruginosa*. The analyses were performed following the Standard Operating Procedures (SOP) identified in the associated Quality Assurance Project Plan (QAPP) developed by OEA with the assistance of the USEPA Region 10 Laboratory. This report is relevant for the following samples:

Sample numbers 08290300 – 0317; 08300300 – 0317.

1.0 Sample Analysis and Determination of Results
1.1 6” x 6” squares of dry suit diving material were disinfected and allowed to air dry prior to use in this study.
1.2 A standard aliquot of *Pseudomonas aeruginosa* containing approximately 10,000 cfu was applied to the swatches of pre-cleaned dive suit material. The level of bacteria in the standard aliquot was determined directly with each separate analytical run by testing in triplicate. Data table refers to column A (D2).
1.3 The number of organisms recovered from the dive suit material after a 3 or 1 minute contact time on the dive suit material in the absence of the disinfectant was used as the bacterial load in the applied aliquot, or the “results per 100 ml.” This was also done in triplicate and results were averaged to obtain the level demonstrated in the report.
1.4 Triplicate samples of dry suit material were used to determine the effectiveness of a 4.7 % solution of betadine at reducing the number of bacteria present on the dive material within a timed exposure period. This was also done in triplicate for each set of data. Data table refers to log removal and percent removal.

2.0 Quality Control Tests Performed:
As established in the Quality Assurance Project Plan for this project, the following quality control tests were conducted as an integral part of these analyses:
2.1 Negative control – triplicate sets of pre-cleaned dive suit material was rinsed with buffer and the residue collected and filtered to check for background *Pseudomonas* contamination. A positive result (growth) from a negative control would have invalidated the data associated with that set.

Negative filtration control – Filtration of 100 ml of sterile rinse water performed to ensure that the filtration portion of the analysis demonstrates no bacterial contamination. Filters are placed on media and incubated with the test samples. A positive result (growth) on this control invalidates the data associated with the set.

Positive control – Standard aliquots of *Pseudomonas* culture were applied to triplicate swatches of dive suit material. After the timed interval without exposure to the disinfectant, the dive suit material was rinsed; with the rinsate being directly filtered through 47 mm diameter, 0.45 µm porosity filters. The filters were placed on mPA agar and incubated. The number of organisms counted and factoring in the dilution used, the number obtained was used in calculations determining the percent removal or log removal of organisms during the disinfection step. A negative result (no growth) on this control would have invalidated the data associated with this set.

3.0 General Conclusions and Disclaimers:

3.1 For this study, two time exposure intervals were utilized to determine effectiveness of the disinfectant at reducing the levels of viable organisms. Three minutes was used initially, but once it was realized that essentially 100 % of organisms were rendered nonviable at three minutes, the study was expanded to include one minute exposures. Although the results were somewhat lower for the three minute exposure (more organisms viable), both time intervals resulted in greater than 3 log (99.9 %) reduction in viable organisms and the results are not statistically significant.

3.2 Objectives to study:

1) **Determine the efficacy of the potable water rinse procedure for removal of bacteria on the Viking drysuit material.**
   a. By comparison of column D2 (initial seeding) and column C in all sets of data, it is apparent that a potable water rinse will effectively remove up to 100 % of the organisms, although there is a range of removal from 74 – 100 % (rsd 13.3 %). However, the organisms rinsed off the dive suit were viable and could present a biological hazard on board the vessel or in the waterway.

2) **Determine the efficacy of Betadine to kill bacteria present on the Viking drysuit material. Two different application periods (1 minute and 3 minutes) will be used.**
   a. The data compiled from 3 sets of triplicate studies demonstrated a 99.98 % (rsd = 0.15 %) reduction of viable organisms using 4.7 % betadine solution and a 3 minute exposure time.
   b. The data compiled from 3 sets of triplicate studies demonstrated a 99.99 % (rsd = 0.006 %) reduction of viable organisms using a 4.7 % betadine solution and a 1 minute exposure time.

3) **Determine the efficacy of hydrogen peroxide to kill bacteria present on the Viking drysuit materials. Two different application periods (1 minute and 3 minutes) will be used.**
   a. Not yet completed.
4) **The primary intent is to remove bacteria from the suit; absent this, the intent is to kill bacteria in-situ.**

   a. See discussion in section 1 and 2 above. Although the organisms are readily removed from the dive suit using a potable water rinse, the organisms are viable at this point and could represent a safety risk or a source of contamination to the water way.

Abbreviations used in data report:

3.7.1 ND - Analysis Not Done
3.7.2 Cfû – colony forming units (basically number of bacteria, assuming a single colony represents a single bacteria)
Quality Assurance Project Plan
for
Viking Dive Suit Decontamination Study
EPA Region 10

Prepared by
Environmental Services Unit
Office of Environmental Assessment

July 9, 2008

/s/__________7/9/08________________________
Dr. Stephanie Harris, EPA Region 10/MEL Sr. Microbiologist/Project Lead Date

/s/__________7/9/08________________________
Rob Pedersen, EPA Region 10 Project Officer Date

/s/__________7/9/08________________________
Ginna Greppo-Grove, EPA Region 10 Acting QA Manager Date

Keven McDermott, EPA Region 10 ESU Manager Date
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<td>11</td>
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<td>11</td>
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1.0 Project Management

1.1 Distribution List

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1.2 Project Organization

(EPA QA/R-5 A4)

The following individuals are responsible for design and implementation of this project, and/or will be the primary data users and decision makers:
Stephanie Harris, (360) 871-8710, EPA Region 10 Environmental Laboratory investigator, is responsible for assisting with the preparation of the quality assurance project plan (QAPP), analysis of samples, and preparation of the final report.

Rob Pedersen, (206) 553-1646, Project Manager, will serve as the primary point of contact for the project.

1.3 Problem Definition/Background

(EPA QA/R-5 A5)

1.3.1. Background

The EPA Region 10 dive team members work in polluted water conditions – contaminated water diving (CWD) from both biological and chemical hazards. Examples are diving near wastewater treatment plant outfalls, storm water outfalls, seafood processor waste, and general chemical contaminates in the water column and sediment. The divers’ suits are made of vulcanized rubber for easier decontamination (Viking Pro EPDM/natural rubber (1000 gr/m²)).

Decontamination is intended not only to rid the diver of bottom sediments, but to dilute and rinse off microbial contamination; Reference: EPA Diving Safety Manual, Appendix L. Diver dress is typically an AGA full face mask (mated to the hood of the viking drysuit), dry gloves, a Viking drysuit, a buoyancy compensator, and SCUBA equipment. See http://yosemite.epa.gov/r10/oea.nsf/webpage/dive+team, Equipment page for more details. This diver dress renders the diver completely dry during contaminated water diving, absent suit punctures or fit issues with the full face mask. Though the improperly equipped diver with a wetsuit and bite mouth regulator is exposed during the dive itself, EPA diver exposure during a dive operation would be more likely after doffing contaminated equipment that has been inadequately decontaminated. Investigation as to the adequacy of potable water rinse as the day to day decontamination technique is needed to determine whether this is sufficient, or if more elaborate antimicrobial decontamination is worth the additional time and effort to employ. Antimicrobial solutions take significantly more effort to deploy with SCUBA equipment due to the limited air supply carried by the diver. If the diver must spend a significant amount of time performing decontamination, this is time that cannot be spent doing work diving. Time may also become a hazard if the diver is overheating in the sun.

Many heavily contaminated water diving operations are conducted with surface supply to provide unlimited air for decontamination. However, surface supply operations severely limit the work the diver can undertake without relocating the dive platform a number of times. For long transect dive surveys, use of a surface supply system therefore becomes impractical. For these reasons, it is in the divers’ interest to have decontamination that is both very effective and of the shortest duration possible.

Decontamination of divers occurs as they exit the water onto the swim step on the stern of the vessel (exclusion zone). Decon generally consists of a thorough potable water rinse. If exposure occurred with a known high biological hazard, then the divers may be sprayed with a diluted Betadine solution (povidone-iodine) as a disinfectant. Betadine is the name of Purdue Pharma’s brand of consumer-available povidone-iodine (PVPI) topical antiseptics. Betadine, is available as a solution, sold over-the-counter (OTC) for cleaning minor wounds[1] and used in hospitals to prepare a patient's skin prior to surgery.[2] Solutions are 10% povidone-iodine in water. A contact time of two minutes is recommended for some disinfection uses with the Purdue Pharma’s product. The Betadine solution used by the dive
team for soaking AGA masks and for Viking suit decon-spray is 9 oz. Betadine to 1.5 gal. freshwater (a 4.7 percent solution).

A common disinfectant is diluted chlorine bleach. This substance is corrosive to silicone and vulcanized rubber as is not used by the dive team. Hydrogen peroxide (H₂O₂) may be an effective disinfectant for use on the Viking suits. The common household hydrogen peroxide is ten percent by volume.

Information on hydrogen peroxide:

10% (v/v) Aqueous Solution (Approximately 3% by weight)

Corrosiveness of hydrogen peroxide

The corrosiveness of process water due to hydrogen peroxide depends on the amount of dissolved oxygen that is produced. Oxygen corrodes iron-containing metals. The amount of iron and the pH are a greater influence on corrosiveness than the concentration of hydrogen peroxide is.

How is hydrogen peroxide transported and stored?

Hydrogen peroxide must be transported in polyethylene, stainless steel or aluminium containers. When hydrogen peroxide comes in contact with flammable substances, such as wood, paper, oil or cotton (cellulose), spontaneous ignition may occur. When hydrogen peroxide is mixed with organic matter, such as alcohols, acetone and other ketones, aldehydes and glycerol, heavy explosions may occur. When hydrogen peroxide comes in contact with substances, such as iron, copper, chromium, lead, silver, manganese, sodium, potassium, magnesium, nickel, gold, platinum, metalloids, metal oxides or metal salts, this may result in powerful explosions. This is why hydrogen peroxide is usually transported in diluted form.

Is hydrogen peroxide efficient?

The efficiency of hydrogen peroxide depends on several factors, such as pH, catalysers, temperature, peroxide concentration and reaction time.

For reactivity to Viking suit material, the manufacture’s chemical permeation test results were consulted.

From Viking Dry Suits CD-ROM:
Sodium hydroxide is an oxidizer similar to hydrogen peroxide. The table above shows a breakthrough time of greater than three hours in a ten percent solution of NaO₂. There was no effect on the suit seams as shown in the table below.

**Table #2: Chemical Permeation Test Results on**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration %</th>
<th>Solubility in Water</th>
<th>Specific Gravity</th>
<th>Breakthrough Time Minutes</th>
</tr>
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<tbody>
<tr>
<td>Acetone</td>
<td>10</td>
<td>100</td>
<td>0.79</td>
<td>PRO 50 HD 60 Latex 90</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>10</td>
<td>100</td>
<td>0.78</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Ammonia Solution</td>
<td>10</td>
<td>100</td>
<td>*</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Carbon Disulphide</td>
<td>100</td>
<td>0.2</td>
<td>1.26</td>
<td>1 1 8</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>100</td>
<td>1.3</td>
<td>1.34</td>
<td>5 5 17</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>10</td>
<td>82</td>
<td>0.71</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>10</td>
<td>100</td>
<td>0.95</td>
<td>&gt;180 &gt;180 &gt;180</td>
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<tr>
<td>Ethyl Acetate</td>
<td>8.7</td>
<td>8.7</td>
<td>0.9</td>
<td>20 52 65</td>
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<tr>
<td>Hexane</td>
<td>0.014</td>
<td>0.014</td>
<td>0.66</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Methanol</td>
<td>10</td>
<td>100</td>
<td>0.79</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>10</td>
<td>50</td>
<td>2.13</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Sulphuric Acid</td>
<td>10</td>
<td>100</td>
<td>1.83</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>0.015</td>
<td>0.015</td>
<td>1.62</td>
<td>&gt;180 &gt;180 40</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>10</td>
<td>100</td>
<td>0.89</td>
<td>60 &gt;180 80</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.05</td>
<td>Not soluble</td>
<td>0.97</td>
<td>&gt;180 &gt;180 85</td>
</tr>
<tr>
<td>Oil No. 1 acc. to ISQ 1497**</td>
<td>100</td>
<td>Not soluble</td>
<td>&lt;1</td>
<td>&gt;180 &gt;180 &gt;180</td>
</tr>
</tbody>
</table>

**Table #3: Diffusion through Seams for Viking Suits**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration in Water</th>
<th>Solubility in Water</th>
<th>Specific Gravity</th>
<th>Diff G/HR</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Acetone</td>
<td>10</td>
<td>100</td>
<td>0.79</td>
<td>4.8</td>
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<td>Acetonitrile</td>
<td>10</td>
<td>100</td>
<td>0.78</td>
<td>0.02</td>
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<tr>
<td>Ammonia Solution</td>
<td>10</td>
<td>100</td>
<td>*</td>
<td>0.01</td>
<td>No effect</td>
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<tr>
<td>Dichloromethane</td>
<td>100</td>
<td>1.3</td>
<td>1.34</td>
<td>48</td>
<td>Reversible swell</td>
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<tr>
<td>Diethylamine</td>
<td>10</td>
<td>82</td>
<td>0.71</td>
<td>0.36</td>
<td>No effect</td>
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<tr>
<td>Dimethylformamide</td>
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<td>100</td>
<td>0.95</td>
<td>0.02</td>
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<td>Ethyl Acetate**</td>
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<td>8.7</td>
<td>0.9</td>
<td>0.01</td>
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<tr>
<td>Ethyl Acetate**</td>
<td>100</td>
<td>8.7</td>
<td>0.9</td>
<td>4.1</td>
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<td>Hexane**</td>
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<td>0.014</td>
<td>0.66</td>
<td>29</td>
<td>Moderate reversible swell</td>
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<td>Methanol</td>
<td>10</td>
<td>100</td>
<td>0.79</td>
<td>0.25</td>
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<td>10</td>
<td>50</td>
<td>2.13</td>
<td>0.01</td>
<td>No effect</td>
</tr>
<tr>
<td>Sulphuric Acid</td>
<td>10</td>
<td>100</td>
<td>1.83</td>
<td>0.02</td>
<td>No effect</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>0.015</td>
<td>0.015</td>
<td>1.62</td>
<td>0.03</td>
<td>No effect</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>10</td>
<td>100</td>
<td>0.89</td>
<td>0.05</td>
<td>Reversible swell</td>
</tr>
<tr>
<td>Tetrahydrofuran**</td>
<td>100</td>
<td>100</td>
<td>0.89</td>
<td>27.5</td>
<td>Seam starts to delaminate</td>
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<tr>
<td>Toluene**</td>
<td>.05</td>
<td>.05</td>
<td>0.87</td>
<td>0.04</td>
<td>No effect</td>
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The required contact time for Betadine to be an effective disinfectant under the conditions the dive team experiences on the back of the EPA vessel is not well known. For other topical disinfectant applications with Betadine, contact times of up to three minutes are mentioned. It may also be necessary for the Betadine solution to dry after application.

Given the reaction of hydrogen peroxide to biological substances, a relatively short contact time may be sufficient. Also, drying in-place on the diver’s suit may not be necessary for hydrogen peroxide. The diver team has not used hydrogen peroxide for disinfection purposes.

This decon study will compare the effectiveness of freshwater rinsing of Viking suit material to rinsing suit material after a one minute and a three minute exposure to Betadine, and after a one minute and a three minute exposure to ten percent hydrogen peroxide.

1.3.2 Objectives and Goals
The objectives of the decontamination procedure study are to:

1) Determine the efficacy of the potable water rinse procedure for removal of bacteria on the Viking drysuit material.
2) Determine the efficacy of Betadine to kill bacteria present on the Viking drysuit material. Two different application periods (1 minute and 3 minutes) will be used.
3) Determine the efficacy of hydrogen peroxide to kill bacteria present on the Viking drysuit materials. Two different application periods (1 minute and 3 minutes) will be used.
4) The primary intent is to remove bacteria from the suit; absent this, the intent is to kill bacteria in-situ.

Information from this study will be used to modify diver decontamination procedures. Results will also be presented to the EPA National Diving Safety Board and peer groups.

1.4 Project/Task Description and Schedule
This QAPP provides the supportive information used in developing the study plan for laboratory methods testing of diver decontamination procedures.

Project Schedule

1.5 Data Quality Objectives and Criteria
Data from this phase of the project will be used as background to develop further data quality objectives for this project.

1.5.1 Objectives and Project Decisions
The primary data quality objective for the diver suit microbial source decontamination study is to characterize the effectiveness of three decontamination procedures. If a potable water rinse is effective at removing relatively high concentrations of bacteria on Viking suit material, then antimicrobial use
may be unnecessary for the majority of Region 10 CWD exposures (thought to be at relatively low concentrations of bacteria).

1.6 Special Training and Certification
The Region 10 Environmental Laboratory staff has completed the required annual 8-hour health and safety training. The Region 10 Laboratory is accredited by the National Environmental Laboratory Accreditation Council. The Region 10 Environmental Laboratory analysts have the appropriate training to conduct bacteriological analyses.

1.7 Documents and Records

1.7.1 QAPP Distribution
It will be the responsibility of the project manager to ensure that appropriate project personnel have the most current, approved version of the QAPP, including updates. The final version of the QAPP and any updates will be distributed in portable document file format.

1.7.2 Determination Levels
Concentrations of inocula to be placed on the Viking drysuit material to test the efficacy of the disinfectant will be determined by previous serial dilution testing. Fresh cultures of *Pseudomonas aeruginosa* (ATCC 27853) will be used each test day. A minimum of $10^4$ organisms will be placed on the suit patch in order to ensure that a 99.0 % (2 log) removal can be determined.

Method detection limit is 1 CFU (colony forming unit) per 100 ml.

1.7.3 Measurement Performance Criteria/Acceptance Criteria
The measurement performance criteria/acceptance criteria for this project are discussed in Section 2.4, Quality Control. In general, if a sample, or associated controls, fall outside of the acceptance criteria, they are invalidated and either re-sampled or re-analyzed, as appropriate.

1.7.4 Laboratory Documentation and Records
Laboratory documentation will include but is not limited to raw data, sample preparation and analysis logs, and results of calibration and quality control (QC) checks.

1.7.5 Quarterly and/or Final Reports
The EPA Region 10 Laboratory will archive the electronic and hard copies of analytical data for a minimum of 10 years as per the QA manual.

2.0 Data Generation and Acquisition
The elements in Sections 2.1-2.10 ensure that appropriate methods for sampling, measurement and analysis, data collection, data handling, and quality control (QC) activities are employed and documented.

2.1 Sampling Design (Experimental Design)
This outline of the laboratory procedure will occur twice, once with Betadine and once with hydrogen peroxide:

2.1.1 Use 6”X6” patches of Viking suit material.
2.1.2 Clean the patches (soap/water, disinfect, air dry).
2.1.3 4 patches placed on a clean surface.
2.1.3.1 Patch 1 inoculated by swabbing with *Pseudomonas aeruginosa* (control patch - this will also catch any potential background that remains on the patches).
2.1.3.2 Patches 2-4 inoculated by swabbing with *Pseudomonas aeruginosa* (will have a known concentration of *Pseudomonas* - this is part of the purpose of patch 1).
2.1.3.3 Allow patches to partially dry (5 minutes), but not completely.
2.1.4 Hang patches over clean pans or beakers.
2.1.5 Decon:
   2.1.5.1 Patches 1-2, rinse with sterile water (patch 1 will be the control and patch 2 a “test” for sterile water rinsing as appropriate for removing the bacteria).
   2.1.5.2 Patches 3-4, spray with Betadine solution (dilution ratio is 9 oz. Betadine to 1.5 gal. freshwater) for decon test one, spray with ten percent hydrogen peroxide for test two.
   2.1.5.3 Patch 3, rinse with sterile water after 1 min. of Betadine/or H$_2$O$_2$ exposure.
   2.1.5.4 Patch 4, rinse with sterile water after 3 min. of Betadine/or H$_2$O$_2$ exposure.
2.1.6 Filter a volume of the rinsates (usually 100 - 500 ml) through a 0.45 um porosity 47 mm diameter membrane filter.
2.1.7 After rinsing the filtration funnel, apply the membrane on a poured plate of mPAC agar so that there is no air space between the membrane and the agar surface.
2.1.8 Invert plates and incubate at 41.5 +/- 0.5 degree C for 72 hours.
Media - m PA - C (modified m-PA agar) available commercially. Media will be prepared to manufacturer's requirements.
2.1.9 Count colonies and report as number of *P. aeruginosa*/100 ml.
2.1.10 Determine percent “kill” in bacteria count (on a log basis) in rinsates 2-4 relative to rinsate 1, and between rinsate 2 with rinsates 3-4.
Note: there will be up-front work to determine the amount of inoculum to place on the patches. In order to have log removals, it is necessary to start with large numbers of organisms and then perform dilutions with the inoculum to obtain countable numbers.
2.1.11 Repeat the decon test at least twice more, on different days.
2.1.12 Determine if we there is a statistically significant difference between rinsates 1-2 with rinsate 3 and rinsate 4 (and also between 3 and 4). If there are an adequate number of samples, the analysis method might be a series of paired T-tests (for homoscedastic data) or multivariate analysis for non-normal data.

### 2.2 Laboratory Procedures

Analytical methods, expected range of results, and required detection limits are summarized in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Method</th>
<th>Lab</th>
<th>Sample Container</th>
<th>Preservation</th>
<th>Holding Time</th>
<th>Precision/Quantitation Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em></td>
<td>Membrane filtration</td>
<td>APHA 9213E EPA</td>
<td>NA</td>
<td>NA</td>
<td>8 hours</td>
<td>20% RSD* / 1 cfu/100 mL</td>
<td></td>
</tr>
</tbody>
</table>

* RSD - Relative standard deviation, standard deviation divided by the mean

**Table 4 analytical Methods Summary**

### 2.2.1 Health and Safety
When working with potentially hazardous materials, investigators are to follow USEPA, Occupational Safety and Health Administration and site-specific health and safety procedures.

2.3 Analytical Methods - Standard Methods (APHA) 9213E (Membrane Filtration Method for *Pseudomonas aeruginosa*).

2.4 Quality Control (QC)
The following QC activities will be performed by the laboratories performing analytical services in support of this project.

2.4.1 Samples Analyzed by the EPA Region 10 Laboratory

Replicate Analysis (Analyst and Method Precision):
Replicate analyses will occur with 10% of laboratory generated samples. Analyst precision will be determined by duplicate counts of the same plate. Analysts must be within 10% for two analyst counts and 5% for single analyst counts.

Method Accuracy:
Negative culture controls (non-target organism) will be analyzed on a daily basis to ensure that the media used for the evaluation is appropriately restrictive for growth of non-target organisms. Positive culture control organisms (target organism) will be analyzed on a daily basis to ensure that the media is appropriate for the organism’s growth.

2.5 Instrument/Equipment Testing, Inspection, and Maintenance

2.5.1 Field Measurement Instruments/Equipment
This phase of the project requires no field instruments.

2.5.2 Laboratory Analysis Instruments/Equipment
Laboratory instruments such as incubators, pH meters and other equipment required by the applicable analytical methods will be maintained according to the manufacturers’ instructions and the Laboratory SOPs. Records for equipment service shall be maintained by the Laboratory.

2.6 Instrument/Equipment Calibration and Frequency

2.6.1 Field Measurement Instruments/Equipment
No field instruments will be used during this phase of the project.

2.6.2 Laboratory Analysis Instruments/Equipment
Laboratory equipment (e.g. pH meter, incubator, etc.) will be calibrated using the method and frequency specified in the Laboratory’s SOPs. Records on calibration of laboratory equipment shall be maintained by the Laboratory.

2.7 Inspection/Acceptance Requirements for Supplies and Consumables

2.7.2 Laboratory Analyses Supplies and Consumables
The quality of chemicals, media and other supplies and consumables used in the Laboratory is dictated by the sensitivity and specificity of the analytical techniques being used. In the Region 10 Laboratory, chemicals, media, and other supplies and consumables are marked with the date received and the receiver’s initials. In the event an expiration date has not been assigned by the manufacturer, the
expiration date will be assigned by the receiver according to the Region 10 Laboratory’s work instruction, “General Guidelines for Assigning Standard Expiration Dates.” The quality of all laboratory supplies is documented by the supplier and the Region 10 Laboratory requests and keeps the vendor certificates on record per NELAC requirements.

2.8 Data Acquisition Requirements (Non-Direct Measurements)
Not applicable to this project.

2.9 Data Management
The Laboratory will maintain a logbook that includes the time of analysis and analyst initials. Quality control results will be recorded on bench sheets. All data generated by Region 10 will be subject to a peer review then signed-off by the Microbiology Team Technical Director. Data entry staff will process and distribute all information mentioned above in accordance with the Laboratory’s SOP. Logbooks, bench sheets and final reports will be stored on-site. All data generated during this project will be processed, stored, and distributed according to Laboratory SOPs.

3.0 Assessment and Oversight

3.1 Assessments/Oversight and Response Actions
Laboratories routinely perform performance checks using method-specific positive and negative controls, blind samples, etc. Corrective actions will be implemented in response to any QA results or detection of unacceptable data. These corrective actions will be developed in consultation with the Office of Research and Development, keeping the data user informed of any impacts on the data. If required, corrective actions will be documented in Appendix A-3.2, Corrective Action Form.

3.2 Reports to Management
A final report will be generated at the completion of the project. This report will include a discussion of the findings, interpretation of data and an executive summary. The report will be provided to all individuals listed in section 1.1 (Distribution List).

4.0 Data Review and Usability

4.1 Data Review, Verification, and Validation Requirements

4.1.1 Data Verification/Peer Review
Region 10 data verification and peer review will be accomplished following the Laboratory’s SOP Mi_D001A (Data Review). Data will be qualified as necessary to convey to the user any important information that needs to be considered in its use.

4.1.2 Data Validation
Data validation is an evaluation of the technical usability of the verified data with respect to the planned objectives of the project. This is accomplished by applying a defined set of performance criteria to the body of data in the evaluation process. Data validation for this project will be performed by the project manager.

4.2 Verification and Validation Methods
4.2.1 Data Verification
Verification of the Region 10 analytical results is the responsibility of the Microbiology Technical Director, as required by the Laboratory’s QA Manual. If any deviations are identified, the potential impact of those deviations on the reliability of the data will be assessed, and the information will be provided to the project manager through the QA Memo and appropriate flagging of the data.

4.2.2 Data Validation
Data validation will evaluate all individual samples collected and analyzed to determine if the results are within acceptable limits. Quantitative or qualitative limits of acceptability are defined for precision, accuracy, representativeness, comparability, and completeness.

1) Precision is defined as the agreement between a set of replicate measurements without assumption and knowledge of the true value. Agreement is expressed as either the relative percent difference (RPD) for duplicate measurements or the range and standard deviation for larger numbers of replicates. Data on precision are obtained by analyzing duplicate and replicate samples.

2) Accuracy is a measure of the closeness of a sample analysis result to the “true” value. Accuracy will be determined primarily by an evaluation of the agreement between repeat analyses within the laboratory.

Representativeness is defined as the degree to which data accurately and precisely represents characteristics of a population, parameter variations at a sampling point, or an environmental condition. For this project, representativeness will be ensured by selection of diver suit material and surrogate decontamination techniques in accordance with the sampling design requirements in this QAPP.

3) Data are comparable if collection techniques, measurement procedures, methods, and reporting units are equivalent for the samples within a sample set. Comparable data for this project will be obtained by specifying standard units and using standard procedures for sample processing and analysis.

4) Data are complete when a prescribed percentage of the total intended measurements and samples are obtained. Analytical completeness is defined as the percentage of valid analytical results requested. For this project, acceptable completeness is > 90%.

4.3 Reconciliation with User Requirements
All data and related information obtained during the course of this project will be included in a data report. Presentations of data and data analysis may be made to relevant user groups upon request.
APPENDICES

Appendix A.

Appendix C. Laboratory Documentation

  C-1. QA Manual
  C-2. Standard Operating Procedures
  C-3. Data Report Forms

Appendix D. Data Evaluation
APPENDIX A. DATA QUALITY PROCEDURES

Figure 1-1. Organization

Project Organization: Refer to section 1.2 for project participant roles and responsibilities.

Table A-1. Data Quality Objectives Summary

<table>
<thead>
<tr>
<th>Analytical Group</th>
<th>Number of Samples</th>
<th># of QA Samples: Reference Samples</th>
<th>Matrix</th>
<th>Method</th>
<th>Method Detection Limits</th>
<th>Accuracy</th>
<th>Precision (RPD)</th>
<th>Completeness</th>
<th>Volume, Container</th>
<th>Holdin g Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Pseudomon as aeruginosa}</td>
<td>4 per event</td>
<td>1 each batch</td>
<td>Water</td>
<td>Standard Methods 9213E)</td>
<td>1 CFU</td>
<td>+/- 10%</td>
<td>+/- 10%</td>
<td>&gt; 90%</td>
<td>clean dry suit</td>
<td>6 hours</td>
</tr>
</tbody>
</table>

1 – Standard Accuracy and Precision for analysis by PCR is unknown at this time. Identification is not quantitative. CFU: Colony Forming Unit
### Table A-2. Analytical Parameters and Target Limits

<table>
<thead>
<tr>
<th>Matrix/Media:</th>
<th>Analytical Parameter</th>
<th>Project Action Limit/Level (applicable units)</th>
<th>Laboratory Limits(^1) (applicable units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Quantitation Limits</td>
<td>Detection Limits (if appropriate)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1 cfu/100 mL</td>
<td>1 cfu/100 mL</td>
<td>1 cfu/100 mL</td>
</tr>
</tbody>
</table>

\(^1\) Laboratory quantitation limits and detection limits are those that an individual laboratory or organization is able to achieve for a given analysis on a routine basis.

- Quantitation limits are the minimum concentrations that can be identified and quantified above the detection limit within some known limits of precision and accuracy/bias. It is recommended that the quantitation limit is supported by the analysis of a standard of equivalent concentration (typically, the lowest calibration standard).
- Detection limits are the minimum concentration that can be detected above background or baseline/signal noise of an instrument.

cfu: colony forming unit
# Table A-5.2 Quality Control Requirements for Analyses

Analytical Method/SOP: PCR qualitative

<table>
<thead>
<tr>
<th>QC Sample:</th>
<th>Data Quality Indicator (DQI)</th>
<th>Frequency/ Number</th>
<th>Method/SOP QC Acceptance Limits</th>
<th>Acceptance Criteria/ Measurement Performance Criteria</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>LABORATORY ANALYSIS:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtration control</td>
<td></td>
<td>1 per day</td>
<td>Negative, no DNA</td>
<td>negative, no growth</td>
<td>Data reviewed, decision made based on cause</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>1 per day</td>
<td>Positive reaction</td>
<td>Appropriate media reaction</td>
<td>Data reviewed, decision made based on cause</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>1 per day</td>
<td>Negative reaction</td>
<td>Appropriate media reaction</td>
<td>Data reviewed, decision made based on cause</td>
</tr>
<tr>
<td>Replicate analysis or duplicate count</td>
<td></td>
<td>10 % of samples</td>
<td>Within 20 % RSD. 10 % between analysts; 5 % with one analyst</td>
<td>Appropriate DNA amplification</td>
<td>Data reviewed, decision made based on cause</td>
</tr>
<tr>
<td>FIELD ANALYSIS:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A-3.1. Attachment 1 – Sample Alteration Form

Project Name and Number: _______________________________________________________

Material to be Sampled: _______________________________________________________

Measurement Parameter: _______________________________________________________

____________________________________________________________________________

Standard Procedure for Field Collection & Laboratory Analysis (cite reference):

____________________________________________________________________________

Reason for Change in Field Procedure or Analysis Variation:

____________________________________________________________________________

Variation from Field or Analytical Procedure:

____________________________________________________________________________

Special Equipment, Materials or Personnel Required:

____________________________________________________________________________

Initiators Name: ____________________________ Date: ____________________________

Project Officer: ____________________________ Date: ____________________________

QA Officer: _______________________________ Date: ____________________________
A-3.2. Attachment 2 – Corrective Action Form

Project Name and Number: ____________________________________________________

Sample Dates Involved: ________________________________________________

Measurement Parameter: ________________________________________________

Acceptable Data Range: ________________________________________________

Problem Areas Requiring Corrective Action: ______________________________

Measures Required to Correct Problem: ______________________________________

Means of Detecting Problems and Verifying Correction: ______________________

Initiators Name: __________________ Date: __________________

Project Officer: __________________ Date: __________________

QA Officer: __________________
APPENDIX B. FIELD DOCUMENTATION


Not applicable to this project.

Appendix B-2. Standard Operating Procedures

Not applicable to this project.

APPENDIX C. LABORATORY DOCUMENTATION

Appendix C-1. QA Manual

Laboratory analysis and procedures will comply with the guidelines described in the document entitled, *Quality Assurance Manual for the U.S. EPA Region 10 Manchester Environmental Laboratory (October 2005)*. The QA Manual is available at the following website on EPA’s Intranet (G:\Sops\NELAC 2005 QAM\NELACTable.html). If you are unable to access this document and would like to obtain a copy, please contact Stephanie Harris.
Appendix D-1. Data Evaluation/Documentation Form.

D-1.1. Microbiology Laboratory Data Review/Release Form

Project: ____________________________________  Project Code: ________________________
Sample Numbers
Peer Reviewed by: _________________________
Date: _________________________

Raw Data/Quality Control Check

___ Verify positive and negative culture controls associated with media are satisfactory.
___ Verify media sterility was checked.
___ Check for sample carryover/contamination if membrane filtration method used. Note any deficiencies.
___ Check duplicate analyst counts are within 20%, when applicable.
___ Verify that media was prepared within method specifications.
___ Verify that samples were received and analyzed within the holding time.

Bench Sheet Check

___ Is the data package properly labeled?
   ___ Analyst name
   ___ Sample numbers and project name
   ___ Analytical method used
   ___ Date and time of collection/analysis
___ Verify that there is a bench sheet for each sample listed on the Analysis Required forms.
___ Verify that there is a Data Review Memo written for the project - forwarded to ESAT Data Entry Technician
___ Verify that there is a Data Release Memo for this project - forwarded to ESAT Data Entry Technician

Results

___ Verify that the reported results:
   ___ have appropriate qualifiers assigned
   ___ reflect the correct units
   ___ reflect dilution factors used in the analysis
   ___ were transferred correctly from the bench sheets
   ___ were calculated correctly
July 10, 2008

Reply To
Attn Of: OEA-095

MEMORANDUM


FROM: Donald Matheny, Chemist
Office of Environmental Assessment (OEA-095)

TO: Rob Pedersen, Project Officer
Office of Environmental Assessment (OEA-095)

I’ve completed a review of the above Plan and overall approval is provided. If you have any questions, please call me at (206)553-2599.
MEMORANDUM

SUBJECT: Data Release for Microbiology analysis results from the USEPA Region 10 Laboratory.

PROJECT NAME: Micro Drysuit Decon Study

PROJECT CODE: LAB 502R

FROM: Gerald Dodo, Chemistry Supervisor
Office of Environmental Assessment, USEPA Region 10 Laboratory

TO: Rob Pedersen, Project Manager
Office of Environmental Assessment, USEPA Region 10

CC: Barry Pepich, Laboratory Director
Office of Environmental Assessment, USEPA Region 10 Laboratory

I have authorized release of this data package. Attached you will find the microbiology results for the Drysuit Decon Study samples collected 7/15 – 7/24/2008. This is the last of the data associated with this project. For further information regarding the attached data, contact Stephanie Harris at 360-871-8710.
The following is a quality assurance review for the results from the *Pseudomonas aeruginosa* analyses from the Drysuit Decon Study Project. The analyses were performed by EPA microbiologists at the US EPA Region 10 Laboratory in Port Orchard, WA, following US EPA and Laboratory guidelines.

This review was conducted for the following samples: **08290300 – 0317 and 08300300 – 0317**

1. **Data Qualifications**

Comments below refer to the quality control specifications outlined in the Laboratory’s current Quality Assurance Manual, Standard Operating Procedures (SOPs) and the Quality Assurance Project Plan (QAPP). No excursions were required from the method.

All measures of quality control met Laboratory/QAPP criteria.

The Region 10 Laboratory Quality System has been accredited to the standards of the National Environmental Laboratory Accreditation Conference (NELAC).

2. **Sample Transport and Receipt**

Sample transport and receipt did not apply.

3. **Sample Holding Times**

Sample holding times did not apply.

4. **Laboratory Quality Control**

No qualification was necessary based on laboratory quality control criteria.

All laboratory equipment and supplies used in this analytical procedure met the criteria as set forth in the Standard Operating Procedures (SOP) identified in the associated Quality Assurance Project Plan (QAPP).
All positive and negative control measures demonstrated correct responses for these sets of analyses. No qualifications were required based on quality control measures.

5. Reporting Limits

All sample results that fall below the detection limit are assigned the value of the detection limit with the “<” qualifier attached. No results fell below the detection limit.

6. Changes from Preliminary Data

No changes were made between the preliminary and final data.

7. Data Qualifiers

No qualifications were necessary.

8. Definitions

ND - Analysis not done
cfu – colony forming units (number of bacteria, assuming a single colony represents a single bacteria)
This file contains data that is readable into Lotus, Excel, WordPerfect, or most databases.

You will need access to PKUNZIP or WINZIP to decompress the file. Once “unzipped” there will be one large file (more appropriate for importing into a database) with the project code as the file name. The fields will be in the following order:

<table>
<thead>
<tr>
<th>Project ID</th>
<th>Analyte Code</th>
<th>Sample ID</th>
<th>Result</th>
<th>Units Code</th>
<th>Matrix Code</th>
<th>Sample Type Description</th>
<th>Qualifier</th>
<th>Date Collection End</th>
<th>Version (Date this file was created)</th>
</tr>
</thead>
</table>

There will also be multiple smaller files with names such as “METQ1-1.txt,” “GENSA-1.txt,” “BNASA-1.txt,” etc. These files are meant to be imported into Lotus or Excel. To open select File/Open and select file type TEXT or .TXT.

The naming convention is as follows: SSSSTT-#.TXT
Where:

SSSS: Metals (MET), General (GEN), GCMS(BNA, VOA, BNAT, VOAT), GC(GC)
TT: Sample Data (SA, Blanks (Q1), Matrix spikes/controls (Q2), Duplicates (Q3)
#: If the table size exceeds 256 columns then the files will be split into multiple smaller files with sequential numbering. Lotus and Excel can only handle 256 columns.

Sample information appears in the following order:

- Sample ID
- Sample Description
- Sample Type
- Matrix
- Units

(It will be indicated if a cell contains data of units other than the default.)

Analyte information appears in the following order:

- Parameter ID
- Method Code
- Analyte Code
- Analyte Name

For General (also called Classical) and FASP data, sample information appears down the side. All other data has the sample information appearing across the top.

Any questions/suggestions should be e-mailed to Tony Morris at morris.tony@epa.gov.

Revised: April 1, 2002
<table>
<thead>
<tr>
<th>Sample</th>
<th>Microscope</th>
<th>Quantity</th>
<th>Result 1</th>
<th>Result 2</th>
<th>Result 3</th>
<th>Result 4</th>
<th>Result 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg sample</td>
<td>Pseudomonas aeruginosa</td>
<td>10200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reg sample</td>
<td>Pseudomonas aeruginosa</td>
<td>15733</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reg sample</td>
<td>Pseudomonas aeruginosa</td>
<td>15733</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Sample ID</td>
<td>Project ID</td>
<td>Sample Type</td>
<td>Code</td>
<td>Parameter Code</td>
<td>Analyte Code</td>
<td>Analyte Name</td>
<td>Result</td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
<td>-------------</td>
<td>--------</td>
<td>----------------</td>
<td>--------------</td>
<td>--------------</td>
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</tr>
<tr>
<td>Parameter</td>
<td>Units</td>
<td>Result</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-----------------</td>
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<td>--------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>8000</td>
<td>100ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Project Code:** LAB-502R  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Project Officer:** ROB PEDERSON  
**Account Code:** 0910B10P202BD4C  
**Station Description:** SPIKED #1 RUN  
**Collected:** 7/15/08 0:00:00  
**Matrix:** Liquid  
**Sample Number:** 08290300  
**Type:** Reg sample  

**Analytes(s):**  
*90248* Pseudomonas aeruginosa  
08290300 Reg sample
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Units</th>
<th>Qlfr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>Pseudomonas aeruginosa by membrane filtration (MF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prep Method</td>
<td>Pseudomonas aeruginosa by membrane filtration (MF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analytes(s)</td>
<td>*90248</td>
<td>Pseudomonas aeruginosa</td>
<td>8000 per 100ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Project Code: LAB-502R  Collected: 7/15/08 0:00:00
Project Name: MICRO DRY SUIT DECON STUDY  Matrix: Liquid
Project Officer: ROB PEDERSON  Sample Number: 08290302
Account Code: 0910B10P202BD4C  Type: Reg sample
Station Description: SPIKED #1 RUN

<table>
<thead>
<tr>
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Method: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)
Prep Method: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)
Analyze(s): *90248
Project Code: LAB-502R
Project Name: MICRO DRY SUIT DECON STUDY
Project Officer: ROB PEDERSON
Account Code: 0910B10P202BD4C
Station Description: DISINFECTANT 3 MINUTES

Collected: 7/15/08 0:00:00
Matrix: Liquid
Sample Number: 08290303
Type: Reg sample

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Project Code: LAB-502R  
Project Name: MICRO DRY SUIT DECON STUDY  
Project Officer: ROB PEDERSON  
Account Code: 0910B10P202BD4C  
Station Description: DISINFECTANT 3 MINUTES  
Collected: 7/15/08 0:00:00  
Matrix: Liquid  
Sample Number: 08290304  
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Analysis Date: 7/15/2008 00:00:00  
Prep Date:  
Container ID: N1  
Prep Method: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)
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Project Code: LAB-502R  
Project Name: MICRO DRY SUIT DECON STUDY  
Project Officer: ROB PEDERSON  
Account Code: 0910B10P202BD4C  
Station Description: SPIKED #2 RUN  

Collected: 7/16/08 0:00:00  
Matrix: Liquid  
Sample Number: 08290306  
Type: Reg sample  

Analysis Date: 7/16/2008 00:00:00  
Prep Date:  

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Project Code: LAB-502R
Project Name: MICRO DRY SUIT DECON STUDY
Project Officer: ROB PEDERSON
Account Code: 0910B10P202BD4C
Station Description: SPIKED #2 RUN

Collected: 7/16/08 0:00:00
Matrix: Liquid
Sample Number: 08290308
Type: Reg sample

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**BIO**

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**Project Name:** MICRO DRY SUIT DECON STUDY  
**Project Officer:** ROB PEDERSON  
**Account Code:** 0910B10P202BD4C  
**Station Description:** DISINFECTANT 3 MINUTES  
**Collected:** 7/16/08 0:00:00  
**Matrix:** Liquid  
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**Type:** Reg sample

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<td>Pseudomonas aeruginosa</td>
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**Project Code:** LAB-502R  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Project Officer:** ROB PEDERSON  
**Account Code:** 0910B10P202BD4C  
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**Prep Date:**
### Project Code
- **LAB-502R**

### Project Name
- MICRO DRY SUIT DECON STUDY

### Project Officer
- ROB PEDERSON

### Account Code
- 0910B10P202BD4C

### Station Description
- SPIKED #3 RUN

### Collected
- 7/18/08 0:00:00

### Matrix
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### Sample Number
- 08290313

### Type
- Reg sample

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- **Container ID:** N1
- **Analysis Date:** 7/18/2008 00:00:00
- **Prep Date:**

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Analytes(s): *90248** Pseudomonas aeruginosa 10200 per 100ml
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**Project Details**

- **Project Code:** LAB-502R
- **Project Name:** MICRO DRY SUIT DECON STUDY
- **Project Officer:** ROB PEDERSON
- **Account Code:** 0910B10P202BD4C
- **Station Description:** SPIKED #3 RUN
- **Collected:** 7/18/08 0:00:00
- **Matrix:** Liquid
- **Sample Number:** 08290314
- **Type:** Reg sample
- **Prep Date:**

**Prep Method:** Pseudomonas aeruginosa by membrane filtration (MF)

**Method:** SM9213E

**Analytes(s):** *90248 Pseudomonas aeruginosa
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Project Code: LAB-502R  
Project Name: MICRO DRY SUIT DECON STUDY  
Project Officer: ROB PEDERSON  
Account Code: 0910B10P202BD4C  
Station Description: DISINFECTANT 3 MINUTES  

Collected: 7/18/08 0:00:00  
Matrix: Liquid  
Sample Number: 08290317  
Type: Reg sample

Analytes(s): Pseudomonas aeruginosa  
Prep Method: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)  
Analysis Date: 7/18/2008 00:00:00  
Prep Date: 

Container ID: N1
Project Code: LAB-502R  
Project Name: MICRO DRY SUIT DECON STUDY  
Project Officer: ROB PEDERSON  
Account Code: 0910B10P202BD4C  
Station Description: SPIKED RUN #1  
Collected: 7/24/08 0:00:00  
Matrix: Liquid  
Sample Number: 08300300  
Type: Reg sample

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**Sample Number:** 08300301  
**Account Code:** 0910B10P202BD4C  
**Type:** Reg sample  
**Station Description:** SPIKED RUN #1

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**Method:** Pseudomonas aeruginosa by membrane filtration (MF)

**Prep Method:** Pseudomonas aeruginosa by membrane filtration (MF)

**Analytes(s):** "90248  Pseudomonas aeruginosa  15733  per 100ml"
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**BIO**

Parameter: Pseudomonas aeruginosa  
Method: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)  
Prep Method: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)  

Analytes(s): *90248*  
Pseudomonas aeruginosa  

Collected: 7/24/08 0:00:00  
Matrix: Liquid  
Sample Number: 08300303  
Type: Reg sample  
Station Description: DISINFECTANT 1 MINUTE  

Container ID: N1  
Analysis Date: 7/24/2008 00:00:00  
Prep Date: 
**Project Code:** LAB-502R  
**Collected:** 7/24/08 0:00:00  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Matrix:** Liquid  
**Project Officer:** ROB PEDERSON  
**Sample Number:** 08300304  
**Account Code:** 0910B10P202BD4C  
**Type:** Reg sample  

**Station Description:** DISINFECTANT 1 MINUTE  

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Analytes(s):  *90248*  

Pseudomonas aeruginosa
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Project Code: LAB-502R
Project Name: MICRO DRY SUIT DECON STUDY
Project Officer: ROB PEDERSON
Account Code: 0910B10P202BD4C
Station Description: SPIKED RUN #2

Collected: 7/24/08 0:00:00
Matrix: Liquid
Sample Number: 08300306
Type: Reg sample

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Analytes(s): **90248** Pseudomonas aeruginosa 15733 per 100ml
### Project Code:
LAB-502R

### Project Name:
MICRO DRY SUIT DECON STUDY

### Project Officer:
ROB PEDERSON

### Account Code:
0910B10P202BD4C

### Station Description:
SPIKED RUN #2

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### Collected:
7/24/08 0:00:00

### Matrix:
Liquid

### Sample Number:
08300307

### Type:
Reg sample

### Method:
SM9213E Pseudomonas aeruginosa by membrane filtration (MF)

### Prep Method:
SM9213E Pseudomonas aeruginosa by membrane filtration (MF)

### Analytes:
- **Pseudomonas aeruginosa**

### Container ID:
N1

### Analysis Date:
7/24/2008 00:00:00

### Prep Date:
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**Project Code:** LAB-502R  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Project Officer:** ROB PEDERSON  
**Account Code:** 0910B10P202BD4C  
**Station Description:** SPIKED RUN #2  
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**Type:** Reg sample
**Project Code:** LAB-502R  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Project Officer:** ROB PEDERSON  
**Account Code:** 0910B10P202BD4C  
**Station Description:** DISINFECTANT 1 MINUTE

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**Method:** SM9213E Pseudomonas aeruginosa by membrane filtration (MF)
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**Project Code:** LAB-502R  
**Collected:** 7/24/08 0:00:00  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Matrix:** Liquid  
**Project Officer:** ROB PEDERSON  
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**Type:** Reg sample  
**Station Description:** DISINFECTANT 1 MINUTE

### Analyte Details

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**Container ID:** N1  
**Analysis Date:** 7/24/2008 00:00:00  
**Prep Date:**

---

08300311 Reg sample
## Project Code: LAB-502R
## Project Name: MICRO DRY SUIT DECON STUDY
## Project Officer: ROB PEDERSON
## Account Code: 0910B10P202BD4C
## Station Description: SPIKED RUN #3

<table>
<thead>
<tr>
<th>BIO Parameter</th>
<th>Unit</th>
<th>Units</th>
<th>Qlfr</th>
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</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>per 100ml</td>
<td>15733</td>
<td></td>
</tr>
</tbody>
</table>

**Method**: Pseudomonas aeruginosa by membrane filtration (MF)

**Prep Method**: Pseudomonas aeruginosa by membrane filtration (MF)

**Analytes(s)**: Pseudomonas aeruginosa

**Container ID**: N1

**Analysis Date**: 7/24/2008 00:00:00

**Prep Date**: 7/24/08 0:00:00

08300312 Reg sample
<table>
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<tr>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td>per 100ml</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa by membrane filtration (MF)</td>
<td>Analysis Date: 7/24/2008 00:00:00</td>
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**BIO**

**Parameter**: Pseudomonas aeruginosa

**Method**: SM9213E

**Prep Method**: SM9213E

**Prep Date**: 

**Container ID**: N1

**Analysis Date**: 7/24/2008 00:00:00

<table>
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<tr>
<th>Analytes(s)</th>
<th>Result</th>
<th>Units</th>
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<tbody>
<tr>
<td>*90248</td>
<td>15733</td>
<td>per 100ml</td>
</tr>
<tr>
<td>Parameter</td>
<td>Method</td>
<td>Analytes(s)</td>
</tr>
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<td>--------------------</td>
<td>---------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Pseudomonas aeruginosa by membrane filtration (MF)</td>
<td>*90248</td>
</tr>
<tr>
<td>Parameter</td>
<td>Result</td>
<td>Units</td>
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<tr>
<td>-----------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>per 100ml</td>
</tr>
<tr>
<td>Parameter</td>
<td>Units</td>
<td>Qlfr</td>
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<tr>
<td>-------------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5</td>
<td>per 100ml</td>
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</tbody>
</table>

**BIO**

- **Parameter**: Pseudomonas aeruginosa
- **Method**: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)
- **Prep Method**: SM9213E Pseudomonas aeruginosa by membrane filtration (MF)
- **Container ID**: N1
- **Analysis Date**: 7/24/2008 00:00:00
- **Prep Date**: 

---

**Project Code**: LAB-502R  
**Collected**: 7/24/08 0:00:00  
**Matrix**: Liquid  
**Sample Number**: 08300316  
**Type**: Reg sample  
**Station Description**: DISINFECTANT 1 MINUTE
**Project Code:** LAB-502R  
**Project Name:** MICRO DRY SUIT DECON STUDY  
**Project Officer:** ROB PEDERSON  
**Account Code:** 0910B10P202BD4C  
**Station Description:** DISINFECTANT 1 MINUTE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Units</th>
<th>Qlfr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
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<td>per 100ml</td>
<td></td>
</tr>
<tr>
<td>Lab number</td>
<td>Date of disinfection</td>
<td>Length of disinfection (minutes)</td>
<td>D2 results (initial spike)</td>
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<tr>
<td>------------</td>
<td>----------------------</td>
<td>----------------------------------</td>
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<tr>
<td>week 1a</td>
<td>7/15/2008</td>
<td>3</td>
<td>10,750</td>
</tr>
<tr>
<td>week 1b</td>
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<td>3</td>
<td>10,250</td>
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<td>week 1c</td>
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<td>week 2b</td>
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<td>16,750</td>
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<tr>
<td>week 2c</td>
<td>7/22/2008</td>
<td>1</td>
<td>16,750</td>
</tr>
</tbody>
</table>

Notes: 1 ml of “C” dilution bottle should be 10,000 organisms and D2 should be equivalent to 10 organisms/1 ml or 30 organisms per 3 ml. D = Calculate the percent recovery from the dry suit based on a 1 ml addition of dilution bottle “C”, this step will indicate the efficiency of removal and the concentration that should be used to determine the log “kill” of organisms on the test strip. Eg: see test above. 1 ml of “C” added to strips, calculated level of spike (8 (B)) x 10000 divided by expected (30) = 2667 actual seeded amount (A). Recovery of organisms off dry suit (C) = number counted on MF (B) X volume of rinsate, or using example (17 x 150 ml) = 2550 organisms in total spike. Percent recovery off dry suit by rinsing (D) = number of organisms recovered off dry suit(C)/calculated level of spike (A) x 100. (eg. 2550/2667 x 100= 95.6 %. Percent removal of organism from dry suit = Number of organisms recovered from dry suit before disinfection – number of organisms recovered after disinfection divided by number of organisms before x 100. Eg. (2667 – 0/2667)x 100 = 100%. Convert the percentage removed to log removal by conversion to log base 10. Based on significant figures, the log removal for t