

Appendix E



San Joaquin River Basin Bacteria Monitoring Program Verified SWAMP Compliant 28 August 2007 Updated 7 January 2008

TABLE OF CONTENTS

| | |
|--|-----|
| INTRODUCTION..... | 127 |
| FIELD RUN PREPARATION..... | 128 |
| Bacteria-processing Sheets and sample labels: | 129 |
| Bottles: | 129 |
| Labeling: | 130 |
| Blanks: | 130 |
| PBS Blank Preparation Procedures: | 130 |
| Field Equipment: | 131 |
| Water and food: | 133 |
| Personal protective gear: | 133 |
| Other personal items: | 133 |
| Vehicle check:..... | 134 |
| SAMPLE COLLECTION..... | 134 |
| Bacteria Collection:..... | 134 |
| Duplicates:..... | 135 |
| Before leaving site: | 135 |
| SAMPLE PROCESSING..... | 136 |
| Processing:..... | 136 |
| Equipment: | 136 |
| Equipment Preparation: | 137 |
| Preparation for processing: | 137 |
| Split the duplicate samples:..... | 137 |
| Sample Processing:..... | 138 |
| Transfer samples to tray: | 139 |
| Placing the samples in the incubator: | 139 |
| Laboratory clean-up:..... | 140 |
| Pulling trays:..... | 140 |
| Equipment: | 140 |
| Sample Results Procedures:..... | 141 |
| Duplicate Samples QA Verification | 141 |
| Laboratory Clean-up | 143 |
| BACTERIA MONITORING PROGRAM QUALITY ASSURANCE/QUALITY | |
| CONTROL MEASURES: | 143 |
| Comparison Counting..... | 144 |
| Materials | 144 |
| Procedures | 144 |
| Bacteria Colilert Media | 145 |
| Materials Per Lot: | 145 |
| Set Up: | 146 |
| Processing:..... | 147 |
| Recording: | 147 |
| Incubator..... | 148 |
| Initial Set up..... | 148 |
| Fisher Scientific Isotemp Forced Air Incubator, Model 650F | 149 |

| | |
|--|-----|
| Safety concerns..... | 149 |
| Setting the temperature..... | 149 |
| Calibrating the offset of the Fisher Scientific Isotemp Forced Air Incubator, Model 650F | 150 |
| QA Specifications:..... | 150 |
| Troubleshooting..... | 150 |
| Binder BD53 Incubator | 152 |
| Safety Concerns..... | 152 |
| Calibrating the thermostat..... | 153 |
| Setting the temperature..... | 153 |
| Barnstead/Thermolyne Corp. Type I42300 Incubator:..... | 154 |
| Safety Concerns..... | 154 |
| Setting the temperature..... | 154 |
| QA:..... | 155 |
| Incubator QA Performed with each run | 155 |
| Materials: | 155 |
| Cleaning..... | 155 |
| Thermometer..... | 155 |
| Materials: | 156 |
| Procedure:..... | 156 |
| Sealer | 156 |
| Materials: | 156 |
| Procedures:..... | 157 |
| Tray Seal Check..... | 157 |
| Sealer Cleaning | 157 |
| UV Lamp..... | 158 |
| Materials: | 158 |
| Procedures:..... | 158 |
| REFERENCES | 160 |

Appendices

- Appendix A. Sample Field and Bacteria Processing Worksheets**
- Appendix B. Equipment Checklist**
- Appendix C. Comparison Counting Worksheet**

List of Tables

| | |
|---|-----|
| Table 1 Bottle Sizes Based on Type of Sample..... | 129 |
| Table 2 Field Equipment | 131 |
| Table 3 Tool Box and Road Emergency Box Contents | 133 |
| Table 4 Equipment Quality Assurance Measures and Frequency..... | 143 |
| Table 5 Logbook Header - Comparison Counting | 145 |
| Table 6 Logbook Header – Quanticult for Colilert-18 Media..... | 148 |
| Table 7 Fisher Scientific Isotemp 650F Troubleshooting | 150 |
| Table 8 Fisher Scientific Isotemp 650F QA Specifications | 154 |
| Table 9 Logbook Header - Thermometer..... | 156 |
| Table 10 Logbook Header - Sealer | 158 |

Table 11 Logbook Header - UV Lamp..... 159

INTRODUCTION

The San Joaquin River Watershed Unit (SJRWU) of the Central Valley Regional Water Quality Control Board (Regional Board) conducts water quality monitoring programs throughout the San Joaquin River Watershed. Monitoring of the Grassland Area was initiated in May 1985 to evaluate the effects of subsurface agricultural drainage on the beneficial uses of the water bodies contained in the study area. The monitoring program and other data assemblages were developed to provide a basis for agricultural regulatory recommendations and to provide evidence for further studies. In accordance with the Basin Plan 5C (State Water Resources Control Board, 1989); compliance monitoring is included in the objectives of the program. In October of 2000, the Surface Water Ambient Monitoring Program (SWAMP) was initiated and provided the much needed funding to continue these monitoring efforts as well as allow for the expansion of monitoring for other constituents, including bacteria, within the Watershed. This manual is a companion to the Procedures Manual for the San Joaquin River Water Quality Monitoring to be utilized by stakeholder groups and other agencies conducting bacteria monitoring using the Colilert-18.

Accuracy and consistency are critical to ensuring validity of the database and any actions or recommendations resulting from the data. To ensure the accuracy and consistency of the data, the procedures presented in this program manual must be followed. The procedures have been laid out in a stepwise format and includes preparation, sampling, processing, and detailed descriptions where needed. This manual also briefly discusses lab and field maintenance, sample tracking, and data entry.

In this program, above all else, field and lab personnel safety is more important than any sample collected. Any concerns with this program or suggestions for improvement should be brought to the attention of the monitoring supervisor.

FIELD RUN PREPARATION

Safety of field personnel and the collection of quality samples rely heavily on field preparation, therefore, it is essential that field preparation be completed with care and accuracy. In addition, it is important to think ahead for any potential problems that may be met in the field such as weather conditions, road conditions, poison oak, ticks, rattlesnakes, hornets, mosquitoes and black widow spiders.

Field preparation is generally assigned to a specific person, however, it is each sampler's responsibility to know what will be needed in the field and inspect the field equipment prior to leaving the office. If there are any special circumstances, such as leaving early or needing special equipment, ample notice must be given to those in charge of preparation so they can prioritize accordingly.

All procedures must be followed in order to preserve the consistency and validity of results and information. Analytical results will only be as good as the sample collected.

The following outlines the standard preparation procedures for each sampling run:

1. Fill out the field sheet and bacteria-processing sheet (samples of blank and completed sheets can be found in Appendix A).
2. Fill out labels for Quanti-trays
3. Count out the number of each bottle type needed
4. Retrieve the sampling bottles and an appropriate sized ice chest to hold them.
5. Premark the sampling bottles according to the completed bacteria-processing sheet.
6. Fill travel and lab blanks.
7. Assemble field equipment
8. Check the vehicle's tires, oil, etc. before leaving to avoid delays out on the road.

Details for each procedure are given below:

Bacteria-processing Sheets and sample labels:

Bacteria samples require a field sheet, processing worksheet and labels to be filled out during field run set up. These sheets contain space for the sample id, sample processing date, samplers, sample identification, Chain of Custody, and QA/QC samples. For consistency and to ensure non-biased laboratory analyses, samples are identified using one of the sampler's initials, date of collection, and a numbering and lettering system, as follows:

- INTYYMMDD-# Bac
- INT=sampler's initials
- YY=year code (example: 2006=06)
- MM=month code (example: January=01)
- DD=date code (day of the month)
- #=arbitrary number assigned to each site, normally 10 through 60
- Bac=bacteria

Duplicate samples are used for quality assurance programs. The monitoring program supervisor will determine the duplicate sample locations prior to set up. Duplicate sample locations will be identified in the QA sample sections of the field sheet and the bacteria processing worksheet.

Labels are made by copying the sample ID's from the field sheet onto Avery 5160 labels, which are then set aside for later processing.

Bottles:

Retrieve bottles from SJRWU laboratory drawers along with an ice chest(s) in which the bottles can all fit **standing up**. All bottles are factory sealed and sterilized polyethylene bottles.

Table 1 Bottle Sizes Based on Type of Sample

| Bottle Size | Type of Sample | Frequency |
|-------------|-----------------|----------------------|
| 120 ml | Normal Samples, | Each |
| | Field Blank | 1 Per Run |
| | Lab Blank | 1 Per Run |
| | Field Duplicate | 1 Set Per 10 samples |
| 290 ml | Lab Duplicate | 1 Set Per 10 samples |

- For lab duplicate samples, 1-290 mL bottle and 2-120 mL bottles will be prepared.
 - The field sample will be collected in the 290 mL bottle.
 - The 2-120 mL bottles will be used to split the 290 mL bottle during sample processing.

- If the samples will be processed by the same organization collecting the samples, the 2-120 mL bottles will remain in the lab, sealed.
- If the samples will be processed by a different organization, the 2-120 mL bottles will remain sealed and be shipped with the full sample bottles the CVRWQCB lab.
- For field duplicate samples 2-120 mL bottles will be collected at the same site.

Labeling:

Write the sample ID on the side of the bottle with a waterproof marker. Write the sample number and suffix on the cap so both the sampler and those processing the collected samples can easily read it. Mark lab duplicate bottles with the normal and duplicate sample ID numbers.

Place the bottles **standing up** in the ice chest(s) in order (either by number or site). Ensure bottles are arranged so they will not tip over and that water from melted ice cannot seep in under the cap.

Include extra empty bottles for potential problems that may arise and to keep the sample bottles from tipping while in transit.

Blanks:

Travel and lab blanks are included with every run where bacteria samples are collected. Travel blanks are with the samples at all times in the coolers. Lab blanks are processed with travel blanks but are left in the laboratory refrigerator until sample processing and transfer. Phosphate Buffered Solution (PBS) should be used when available for blanks. If unavailable, de-ionized water can be used.

For making bacteria blank, see below (Be sure to wear nitrile gloves and a lab coat):

PBS Blank Preparation Procedures:

(Note this procedure is used when bacteria blanks processed in a 30-day period are sufficient to justify mixing our own. When mixing our own, we will obtain Type II water from the Tate Lab in the LAWR department at UC Davis campus and media from Sigma-Aldrich, item # P3813-10PAK, Phosphate Buffered Saline Powder, pH7.4 (lit.). When we are not mixing our own, we will obtain PBS from the Tate Lab.)

1. Remove shrink-wrap from the sample bottle.

2. Shake PBS bottle to ensure homogenous mixture.
3. Place PBS bottle and 120 ml sample bottle(s) on the workspace.
4. Sterilize nitrile gloves and workspace with alcohol.
5. Allow alcohol to air dry.
6. Remove caps from both bottles, ensuring not to touch the inside of either cap, and place, with inside down, on the sterilized workspace.
7. Fill 120-ml sample bottle with PBS water to the 100-ml line, ensuring to not touch inside of either bottle.
8. Recap both bottles.
9. Place both bottles in the refrigerator, and label with a Post-It: the acronym "PBS", initials of the person who mixed or obtained the PBS from the Tate lab and the expiration date - 30 days from the date the PBS was mixed or obtained.

Place a note on the small bacteria cooler to remind field crew to grab the bacteria travel blank out of the lab refrigerator before leaving the laboratory.

Field Equipment:

Each sampler is responsible to inform the appropriate personnel of any problems relating to equipment. See equipment maintenance (Appendix D) for detailed instructions for proper use and maintenance of equipment.

The CVRWQCB San Joaquin River Watershed Unit for the bacteria monitoring uses the following list of recommended equipment:

Table 2 Field Equipment

| <u>Equipment</u> | <u>Description</u> |
|---------------------------|---|
| Ag/RB/SWAMP Field Books | The field monitoring books contain summarized monitoring information (e.g. site location maps, contact information, etc.), access keys and emergency information for field personnel. Should there be any changes made to the monitoring schedules, the field monitoring books should be updated as well. Attach the field data sheet to the clipboard with a pencil, extra blank labels, and a photograph sheet of the monitoring sites. |
| Clipboards w/field sheets | |
| Roadside Emergency Kit | |
| Toolbox | Inspect the toolbox and road emergency box to ensure that the appropriate contents are present and in working order (Table 1). |
| First Aid Kit | |

| | |
|---------------------------------------|---|
| Jumper Cables | |
| pH/EC Kit | <p>The pH/EC kit is an ice chest containing items used for QA/QC during field sampling runs. Reorganize this cooler and augment any supplies as needed. The supplies include:</p> <ul style="list-style-type: none"> ▪ Calibration solutions ▪ DI Water ▪ Tap Water ▪ Disposable nitrile gloves (small, medium, large & extra large) ▪ Waterproof marker, ball point pen, pencil, safety glasses, paper towels ▪ Myron L EC/pH meter ▪ Liquid soap |
| Bucket (w/ Rope and Insect Repellant) | A stainless steel bucket (triple rinse) and rope can be used for sampling off a bridge when there is no safe access to the bank. Additionally, the bucket can be used to carry sampling bottles to and from the site. |
| Potable Water | |
| Rain Gear (as needed) | |
| Floatation Vests (1 per sampler) | |
| Camera (charged battery) | |
| Cell Phone (charged) | While in the field, each sampling team must have a phone for emergencies. |
| Map Books of sampling sites and areas | |
| Sample Poles | |
| Shovel | |
| Life vests | Even the best swimmers can succumb to hypothermia. Life vests should be worn when sampling from bridges, boats, unstable banks, or during periods of extremely high flow. |
| Sample Coolers | |
| Polyethylene 5-gal Bucket | |
| Sampling poles | Sampling poles are used to retrieve a sample up to six feet from the bank. The pole used for bacteria sample collection utilizes a clamp to hold the sample bottle. |
| Shovel/spades | Carry a shovel or spade in the truck in case the truck gets stuck and must be dug out. |
| Boots: | Each sampler is responsible for being fit for his or her own rubber boots. Boots or hip waders may be required for |

| | |
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| | wading to sample collection location. |
| Ice | |

Table 3 Tool Box and Road Emergency Box Contents

| <u>Tool Box</u> | <u>Road Emergency Box</u> |
|--------------------------------|----------------------------------|
| Screwdriver, pliers | Flares |
| Flashlight | Reflector triangles |
| Graduated cylinder | Fire extinguisher |
| Duct tape | |
| Utility knife | |
| Chain w/ locks | |
| W-D 40 | |
| Sigma tubing | |
| Toilet paper | |
| Spare C Batteries w/ YSI Cover | |
| Utility Saw | |
| Dessicant | |

Water and food:

Drinking water is necessary to maintain normal bodily functions. Bring plenty of water for a day. During the summer, double the amount of water you bring to avoid dehydration. While in the field, convenience food stores are not always available, therefore, pack a lunch and some snacks for the day. **Wash your hands prior to eating as we are sampling waterways that may contain unknown contaminants.**

Personal protective gear:

It is the responsibility of each sampler to think ahead and watch the weather forecast in order to dress appropriately for field monitoring. Items that you may want to consider bringing from home are:

- Hiking boots
- Heavy coat/sweater/sweatshirt
- Hat
- Warm gloves
- Sunglasses
- Sunscreen

Other personal items:

Money is also helpful for those unexpected necessities.

Double Check: After coolers with samples and the appropriate equipment are set up, check all equipment off the checklist. An example checklist can be found in Appendix B.

Vehicle check:

Check the vehicle's tire pressure, oil, coolant, lights, etc. in order to avoid problems and/or delays while in the field. Report any major maintenance needed to the appropriate personnel and if possible retrieve an alternate truck.

SAMPLE COLLECTION

While in the field you must depend on your own instincts and remember that no sample is worth injury. Therefore, the best rule of thumb is:

WHEN IN DOUBT, GET OUT!!!
Be aware of your surroundings at every site
and remember that safety comes first.

All procedures must be followed in order to preserve the consistency and validity of results and information. Analytical results will only be as good as the sample collected.

Photos should be taken at each site to visually document conditions. If possible, include a marker so that others can reference the site, such as a bridge, and so that future photos can be taken so that they capture the same frame. Photo documentation should occur monthly for ongoing projects and per sampling event for short-term studies.

Bacteria Collection:

Two people will be needed for this procedure; one to collect the sample (Sampler 1) and one to hold the cap (Sample 2). This procedure is used to minimize potential for contaminants to fall in the cap or bottle, and in lieu of uncapping the bottle under the water surface.

1. Using the clamp pole either person can secure the bacteria bottle on the clamp and remove the shrink band from bottle.
2. Sampler 1 will then dunk the clamped bottle into the water three times, downstream of the sample site, to condition the bottle and pole.
3. Sampler 2 will remove the cap from the bottle, ensuring the inside of the cap is kept away from contaminants during the sample collection.
4. Sampler 1 will then collect the sample.

- a. Do not turn the bottle upside down – The bottles contain sodium thiosulfate, which is necessary for neutralizing chlorine that might be in the sample water.
 - b. Tilt the bottle so that the opening is facing upstream.
 - c. If there is debris on the water body surface, create a clear sampling zone by artificially creating a current. Push the bottle forward, horizontally, under the water body surface.
 - d. Collect the sample deep enough so that surface debris is not collected (one to two inches below the surface of the water).
 - e. Fill the bottle as close to the 100 ml mark (or 250ml if duplicate) as possible, without going under the line. Remove excess sample water from the bottle by tilting slowly to dump any extra sample water and then re-check.
5. Sampler 2 then caps the bacteria bottle, and unclamps sample.

Duplicates:

- Duplicate samples are collected to comply with QA/QC programs to ensure the precision of contract laboratories' results. Duplicate samples are submitted blindly along with other samples at a frequency of 1 field duplicate and 1 lab duplicate per 10 samples collected.

Lab duplicate samples are collected in 250 ml sterile polyethylene bottles that contain sodium thiosulphate and split in the laboratory during processing. See bacteria processing for splitting procedure.

Field duplicate samples are collected by collecting 2-125 ml polyethylene bottles at the same site. Note the site that the Field duplicates are collected at can differ from the Lab duplicate site.

Before leaving site:

The following information should be recorded on the field sheet before leaving each site:

Time (24 hour clock) the sample was collected.

Field data, if available (Temperature, pH, Dissolved Oxygen, Electrical Conductivity, Photo Number)

Any relative factors that may influence results such as change in flow (low, medium, or high), diversions or other uses being made of the water, weather, or overland flow.

Double-check the area for any equipment that may have been left or not properly secured for the remaining trip.

Keep all samples on ice until they can be processed in the laboratory.

SAMPLE PROCESSING

To maintain sample integrity and ensure viable laboratory results, appropriate handling of samples after collection is essential. It is necessary to commence sample processing promptly after the collection of samples in case there are any unusual developments or questions in regards to the samples collected, such as excessive amounts of sediment or pungent odors. Note any abnormalities of samples on the bacteria-processing sheet in addition to alerting program staff, as there may be a need for immediate action. Maximum hold time allowed is 6 hours at 4°C for regulatory purposes. For non-regulatory purposes, samples must be processed within 24 hours at 4°C. Samples must be kept in a dark location while they are being held. **All personnel must wear nitrile gloves while handling samples.** Wearing safety glasses is required when processing and pulling bacteria samples.

Bacteria processing is completed in house. Any significant changes (such as changing a procedure or the type of water used for blanks) must be annotated in the Significant Events Log in the front of the bacteria QA/QC binder located in the lab, on the shelf above the sealer. There are three parts for bacteria processing:

1. Processing
2. Pulling trays
3. QA/QC

Each of these steps is elaborated in detail below:

Processing:

Sterile technique is important to reduce cross-contamination between samples as well as for personal protection from possible pathogenic organisms. Wear protective nitrile gloves, lab coat, and goggles during processing. Always wear closed-toed shoes while in the lab. Food and drinks are not allowed in the lab. Always remember to turn on the fume hood.

Equipment:

- Nitrile gloves
- Protective Eyewear
- Lab Coat
- Full sample bottles
- Bacteria processing worksheet (Appendix A)
- Spray Bottle filled with Alcohol
- Bag of 97 Well Quantitrays
- Snap packs of Colilert-18 media (QA/QC checked, see bacteria processing)

Equipment Preparation:

Prior to the field crew returning to the lab after a field run, processor should ensure that the sealer, incubator, and fume hood are turned on. The sealer and incubator take approximately 15 minutes to warm up. However, for a uniform air temperature in the incubator, plan on a general warm up time of 90 minutes.

Bacteria samples processed without delay should be removed from the cooler to warm to room temperature. (If processing samples are delayed to wait for the arrival of a second run, samples should be left in the cooler, with the time of their arrival noted on the bacteria processing form.)

Prior to working with the samples, the processor must wear the necessary safety gear; lab coat, nitrile gloves and UV protected goggles. (The UV goggles are manufactured by UVEX and can be identified by the imprint "UVEX" on the goggle arms. UVEX = UltraViolet Excluded)

Preparation for processing:

1. Ensure that one of the samplers sign the bacteria processing form under the 'Sampler' heading and annotates the date and time the samples arrived in the lab. This will serve as the chain of custody for bacteria samples.
2. Annotate the time processing began along with the name of the processor.
3. Disinfect the work area with alcohol from the spray bottle and let air-dry.
4. Remove the bottles from the cooler. Note for consistency, this action initiates the two-hour processing time window.
5. Set bottles from the cooler and the empty bottles prepared during set up for the duplicate samples on a rack over the sink. Remember to grab the Lab Blank from the refrigerator.
6. Triple rinse outside of the bottles with DI water, if available. If DI water is not available, distilled water can be used.
7. Dry the bottles with a paper towel and line them up on the work area in either ascending or descending order.
8. Gather the antifoam solution bottle and place in the work area.

Split the Lab duplicate samples:

- Note splitting is done prior to processing the batch to ensure all samples are treated the same.
1. Place the corresponding 120ml bottles (Already made in preparation) in front of the corresponding 290ml sample bottle.
 2. Remove the shrink bands from the two 120ml bottles.
 3. Remove the 120 ml bottle caps.

4. Invert the 290ml bottle at least ten times to re-suspend any matter that settled.
5. Pour sample into the 120ml bottles to the 100ml line, alternating bottles with equal amounts of sample in approximately 50 ml increments, and swirl the 290ml bottle between pours.
6. Line up the 120ml bottles with the rest of the sample set.

Sample Processing:

1. Ensure samples are at the correct volume.
 - a. The bottom of the meniscus should be on the 100ml line.
 - b. If the sample is over 100 ml, pour out sample water into the sink until the appropriate amount is in the bottle, being careful to not pour out too much sample. Pour the sample water into cap, if necessary to ensure that sample water can be added back if too much has been poured off . The preference is to have enough sample to fill all Quanti-tray wells.
2. Collect the necessary number of snap-packs. Detach them from one another and place one in front of each bottle.
3. Add the contents of the snap pack to the sample container.
 - a. Tap each snap-pack on the counter to shake the contents to the bottom.
 - b. Loosen the bottle cap, but do not remove it.
 - c. Open the snap pack by holding the foil side toward you and pull the top toward you to break the perforations.
 - A slight tinge (light yellow-brown) may be observed when snap pack is added to the sample
 - In samples with excessive chlorine, a blue flash may be seen when adding the snap pack. If this is seen, consider the sample invalid and discontinue testing the sample. Annotate on the processing worksheet that the blue flash was observed.
4. Remove bottle cap and pour the contents of the snap pack into the sample bottle with the other. Ensure that the snap pack does not touch the bottle and that the entire contents of the pack are emptied into the bottle.
5. Add Antifoam Solution to the sample container.
 - a. Invert the Antifoam Solution bottle 3 times prior to dispensing.
 - b. Add 2 drops of Antifoam to the sample bottle.
6. Recap the bottle.
7. Mix the Media from the snap pack and Antifoam Solution with the sample by swirling the bottle.
8. Repeat steps 1-7 for all samples.
9. Once the media has been mixed with all the samples, continue swirling the bottles until the contents of the snap packs have completely dissolved.

- a. While waiting for the media to dissolve, attach the pre made labels to the Quanti-trays, ensuring nothing comes in contact with the opening of the tray.
- b. Place the Quanti-trays in a stack in the order that corresponds to the bottles, and to the side of the work area.

Transfer samples to tray:

- The sealer is ready once the green light is on.
1. Place the pre-made labels on the back side (paper side) of trays.
 2. Swirl the bottle contents and loosen but do not remove the cap from the bottle.
 3. Hold the Quanti-tray with the label that corresponds to the sample ID open and at a 45° angle, keeping the wells facing upwards.
 4. Remove the cap from the bottle and slowly pour the contents of the bottle down the foil side of the tray so as not to create excess air bubbles.
 5. Hold the tray so it is vertical with the opening upward, and tap it to bump any air bubbles out of the small wells.
 6. Place the tray, small wells first, into the sealer insert.
 - a. Slide the insert into the sealer – release once the sealer starts to pull the insert through.
 - b. Do not push the insert into the sealer once the sealer pulls the insert. Allowing the sealer to pull the insert at its own speed minimizes the chance of empty wells.
 7. Remove the insert and tray from the rear of the sealer.
 - a. Do not pull tray until the sealer has released it from the rollers.
 - b. Stack the trays so they can all be placed in the incubator at the same time.
 8. Repeat steps 2-7 until all trays are sealed.

Placing the samples in the incubator:

- Opening the incubator door must be kept to a minimum to maintain a constant temperature within the incubator.
1. Before opening the incubator to put in the first sample, note the incubator temperature (should be 35°C +/- 0.5) on the processing sheet.
 - a. If incubator is not within +/- 0.5°C, verify the temperature with a second thermometer.
 - i. If second thermometer is also not 35°C +/- 0.5, adjust incubator settings as appropriate.
 - ii. If second thermometer reads differently than 1st
 1. Annotate on the bottom of the bacteria processing worksheet what occurred and which thermometers and incubator was used.

2. Use a different incubator, if available.
3. Notify the Program Manager.
- iii. If another incubator is not available, proceed with using the current incubator, ensuring to annotate the temperature on the processing worksheet and notify the Program Manager.
2. Place the trays, wells facing down, on the counter in 4-6 stacks to be inserted in the incubator as bunches.
3. Arrange the trays in the incubator so they are evenly distributed, wells facing down, and spaced to allow air circulation within the chamber.
4. On the bacteria processing worksheet fill in the name of the person who placed the samples in the incubator, and the date and time the samples were placed in the incubator.

Laboratory clean-up:

1. Disinfect all work surfaces with alcohol.
2. Rinse sample bottles with tap water and dispose of bottles in the garage-recycling bin.
3. Remove nitrile gloves by rolling them inside out and discard them in the trashcan.

Pulling trays:

The bacteria trays are typically pulled after 18 hours incubation and must be pulled between 18 and 22 hours.

Equipment:

- Nitrile gloves
- UV/UVEX Goggles (located in the top drawer to the right of the bacteria processing counter)
- Lab coat
- Bacteria results worksheets
- Black Sharpie Marker
- Comparator (located in the second drawer down to the right of the bacteria processing counter)
- UV Light (located in the third drawer down to the right of the bacteria processing counter)
- UV Viewing Cabinet (located on the shelf above the bacteria processing counter)

Note: Items to watch for.

- More positive fluorescent wells than positive yellow wells.
- Empty wells.

- Number of wells recorded does not exceed 48 small wells and 49 large wells.
- All positive wells are marked.
- All items at the bottom of the bacteria processing worksheet are filled in. This form is the chain of custody and provides information on how each batch was handled.

Sample Results Procedures:

1. Before working with bacteria samples, laboratory personnel should wear the appropriate safety gear: nitrile gloves, plastic UV protective goggles, and white lab coat.
2. Pull the Quanti-trays from the incubator and place them in a stack to one side of the work area. (Note ending temperature.)
3. On the bacteria processing sheet under “Pulled from incubator by” fill in the name of the puller, the date, the time the trays were pulled, and the ending incubator temperature prior to opening the incubator door.
4. Place the UV light in the UV Viewing cabinet. This will automatically place the light at six inches above the trays necessary for proper viewing.
5. View trays under normal indoor lighting to determine total coliform.
 - a. Wells that are positive for total coliform will be yellow in appearance equal to or greater than the yellow of the comparator.
 - b. Using a sharpie marker, mark positive wells with a vertical line (|).
 - c. Write the number of positive wells on the upper right corner of the set of large wells and small wells.
6. View trays under UV lighting to determine *E. coli*. It is recommended that the lights be turned off during the *E. coli* analysis.
 - a. Wells that are positive for *E. coli* will fluoresce equal to or greater than the fluorescence of the comparator.
 - b. Using the sharpie marker, mark positive wells with a horizontal line (-).
 - c. Write the number of positive wells on the lower right corners of the set of large wells and the set of small wells.
7. If it cannot be determined if a well is positive or not, and the trays were pulled at 18 hours incubation, place the tray back in the incubator for an additional four hours. If after that time the well has not increased in the yellow color or fluorescence, then it is negative. If it has increased, it is positive.
8. The total positive yellow and fluorescent wells, empty wells, and false positive wells will then be marked on the bacteria processing worksheet.
Note false positive wells are fluorescent but not yellow.

Duplicate Samples QA Verification

1. Duplicate QA is checked once all sample results are annotated on the bacteria processing worksheet.

- a. In the QA section of the bacteria processing, there is space for one set of blanks and two sets of duplicates: Field and Lab. Annotate the Duplicates and Normal Samples based on the field sheet.
 - b. Run the MPN generator program (Can be downloaded from www.idexx.com/water/quantitray. Look for the blue link that says, “free download”.)
 - i. Information to be entered is the number of large wells and the number of small wells per sample included in QA, as indicated on the field sheet.
 - ii. Once the numbers of large and small wells are entered, the Calculate button will be active. Mash the calculate button for the program to respond with the MPN and Upper and Lower Confidence Limits.
 - iii. Enter results in the corresponding boxes of the bacteria processing worksheet QA section.
 - c. QA verification
 - i. Calculate each set of Normal and Duplicate means
 - ii. Verify the mean is within the confidence limit for the Normal and Duplicate Samples for each set
 - iii. Passing QA
 1. Mean MPN falls within both confidence limits
 2. Transfer QA data to the log sheet in the Bacteria QA binder located in the SJR Unit lab.
 3. Follow distribution in 12, below.
 - iv. QA doesn't Pass
 1. Mean MPN falls outside one or both confidence limits
 2. Verify sample numbers
 3. Verify data entry
 4. If needed, contact the program manager to verify tray counts
 5. Transfer QA data to the log sheet in the Bacteria QA Binder located in the SJR Unit lab.
 6. If tray counts are still outside the passing criteria, flag the data as questionable, pending resolution of SWAMP Laboratory Duplicate QA
2. When finished reading the batch of trays, deposit the used trays in the biohazard trashcan in the lab. The trays must be properly disposed of due to their having been live microorganisms in the samples indicate the potential presence of infectious organisms. The following outlines the procedure used by the San Joaquin River Watershed Unit:
- a. The used trays are taken to the Sacramento Regional County Sanitation District (SRCSD) laboratory to be autoclaved and disposed of at least **monthly**. A map to the wastewater treatment facility can be found at: <<http://www.srcsd.com/srwtp-map.html>>.

- b. Trays should be taken to the lab at the beginning of the week as requested by SRCSD lab staff.
 - c. Regional Wastewater Treatment Plant Phone Number: 916/875-9000
3. On the bacteria processing sheet under “Trays read by” fill in the name of the reader and the date.
4. Processing worksheet distribution:
- a. The original bacteria processing worksheet will go in the binder with the original bacteria processing worksheet, maintained by the program manager
 - b. Copy of the bacteria processing worksheet will go to database entry
 - c. Copy of the bacteria processing worksheet will go to QA entry in the QA Log.

Laboratory Clean-up

- 1. Disinfect all work surfaces and anything that has been touched (i.e., markers, UV light, comparator, etc.).
- 2. Disinfect the inside of the incubator.
- 3. Remove nitrile gloves by rolling them inside out and discard them in the trashcan.

BACTERIA MONITORING PROGRAM QUALITY ASSURANCE/QUALITY CONTROL MEASURES:

The following measures are included in the San Joaquin River Watershed Bacteria Monitoring Program. Logs for each of the measures are kept in a bacteria log that is kept with the bacteria analysis equipment in the lab. Each bacteria-monitoring program should maintain a logbook for QA/QC records.

QA/QC measures are conducted at the frequency specified in Table 3:

Table 4 Equipment Quality Assurance Measures and Frequency

| QA/QC Procedures | Frequency |
|-------------------------|---|
| Comparison Counts | Monthly |
| Quanticult (Media) | Once per lot |
| Incubator | Before samples are placed in and taken out of the incubator |
| Incubator Thermometer | Semiannually |
| Sealer | Monthly |
| UV Lamp | Monthly |
| Lab Duplicate | 1 Set Per 10 samples |

| | |
|-----------------------|----------------------|
| Field Duplicate | 1 Set Per 10 samples |
| Lab and Travel Blanks | Per Run |

Comparison Counting

Bacteria comparison counting is done to ensure consistency of positive well counts between analysts, and that the results generated from bacterial tray counting are reliable. On a **monthly** basis, two analysts will perform counts on the same tray. One of those two analysts will then perform counts with a different analyst the following month and so on.

On a monthly basis, 20% of the analysts will perform counts on one tray. Two analysts will perform comparison counts for the month. One of the two will perform counts with a different analyst the following month and so on. This will continue until everyone has compared counts twice.

- For routine evaluation, repeat counts on 10% of positive samples **at least monthly** and compare the counts with those of other analysts who perform the same analysis.
- Replicate counts by the same analyst should agree within 5% and those between analysts should agree within 10%.
- If results disagree more than the prescribed limit, notify the program supervisor – remedial training may be required.

Materials

Lab Coat
 Gloves
 UV Safety Goggles
 Quanti-Trays to be read
 Plastic Numbered Overlay
 Viewing Cabinet
 UV Lamp
 Comparison Counting Worksheets

Procedures

1. Analysts should wear a lab coat, gloves and UV safety goggles.
2. Spray down the processing area with alcohol and allow to air dry.
3. Place viewing cabinet within the workspace.
4. Place the UV lamp on the viewing cabinet and plug it in.
5. Remove trays to be read from the incubator and place in order, face down on the left side of the cabinet and turn over the first tray.
6. Place the numbered plastic overlay over the wells of the tray to be read.

7. Count the number of positive yellow wells and record the number on the comparison counting worksheet. A sample worksheet can be found in Appendix C.
8. Place the tray in the viewing cabinet and count the number of positive fluorescing wells and record the number on the comparison counting worksheet.
9. Another analyst will then repeat the above procedures.
10. Compare worksheets of the two analysts.
11. Record the results on the Comparison counts log sheet under the Comparison counts tab in the QA/QC Log Book.

Table 5 Logbook Header - Comparison Counting

| Date | Sample ID | Analyst 1 | | | Analyst 2 | | | Percent Accuracy | |
|------|-----------|-----------|---------|----------------|-----------|---------|----------------|------------------|------------------|
| | | Initials | T. Coli | <i>E. coli</i> | Initials | T. Coli | <i>E. coli</i> | % T. coli | % <i>E. coli</i> |

Bacteria Colilert Media

When a new lot of Colilert media arrives in the lab, it is tested for effectiveness using a Quanti-Cult kit that is provided by IDEXX. The kit contains preserved cultures of three species of bacteria, pre-measured re-hydration media contained within vials, and a foam rack for holding the vials during incubation. The kit provides both negative and positive control factors to test substrate performance and staff technique (a blank is added). *Escherichia coli* must have both positive yellow and florescent wells; *Klebsiella pneumoniae* must have positive yellow wells but negative florescent wells and *Pseudomonas aeruginosa* must have all negative yellow and florescent wells. The media must pass the above QA/QC acceptance criteria to be utilized in this program. If the media fails the QA/QC acceptance criteria it is disposed of. Note there may be more than one lot number within a single shipment; therefore snap packs from each lot should be tested. For test procedures see the Bacteria Analysis: Procedures / Changes to Program / QA Measures and Log binder located in the SJRWU laboratory.

NOTE: There may be more than one lot number within a single shipment. Therefore, QA/QC should be performed on snap packs from each different lot.

Preserved Cultures
Escherichia coli
Klebsiella pneumoniae
Pseudomonas aeruginosa

Diagnostic Purpose:
+ Yellow Indicator + Florescent Indicator
+ Yellow Indicator - Florescent Indicator
- Yellow Indicator - Florescent Indicator

Materials Per Lot:

4 – Snap-packs of Colilert-18 from the lot being QA'd
4 – 120 ml IDEXX sample bottles
1 set – Quanticult (1 each of *E. coli*, *K. pneumoniae*, *P. aeruginosa*)
400 ml - PBS
1 set re-suspension vials (3 total)
Bacteria Processing Worksheet
Lab safety gear (gloves, goggles)
Foam rack

Set Up:

*Caution: this kit should only be used by or under the immediate supervision of individuals familiar with aseptic methods and the following procedures. Organisms contained within the kit are known human pathogens and should be handled accordingly.

1. Heat the incubator to 35°C.
2. Put on protective gloves.
3. Disinfect the workspace by spraying it down with rubbing alcohol and allowing it to air dry.
4. Select one 120 mL sterile IDEXX bottle per culture plus one extra to run as a blank and label appropriately.
5. Assign each bottle a sample number containing the analyst's initials, the date, a dash and "01" followed by "EC" for *E. coli*, "KP" for *K. pneumoniae*, "PA" for *P. aeruginosa*, and "BL" for Blank (Example :ARP040609-01EC). Label bottles and processing sheet accordingly.
6. Fill culture-containing IDEXX bottles with approximately 98 mL of PBS water. Fill the blank with 100 mL of PBS water.
7. Place all bottles in the pre-warmed incubator.
8. Remove zip lock bag containing the Quanti-Cult kit from the laboratory refrigerator ensuring not to retouch the refrigerator handle with potentially contaminated hands.
9. Place the zip lock bag containing the kit to the side the workspace and remove the kit.
10. Select the following dehydrated cultures: *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.
11. Select one unused vial containing 2 mL of re-suspension fluid per culture and place within the designated workspace only.
12. Return the kit to the zip lock bag and ensure that the bag is closed.
13. Remove one glove and grasp the zip lock bag with the remaining gloved hand. Use the ungloved hand to open the laboratory refrigerator door.
14. Deposit the zip lock bag inside the laboratory refrigerator with the biohazard label clearly visible.
15. Remove the remaining glove by grasping the cuff and rolling the glove off of the hand, folding the exterior surface inside.

16. Dispose of the glove in the trashcan and wash hands thoroughly
17. Once hands are completely dry put on a fresh set of gloves.

Processing:

1. Place unused vials of re-suspension fluid upright in the pre-warmed incubator (35°C), allowing vials to incubate for 5-10 minutes.
2. Select one culture, remove its vial from the foil packaging and place upright in the workspace.
3. Select one vial of re-suspension fluid from the incubator and remove the blue cap.
4. Transfer the colorless cap of the culture vial onto the vial containing the re-suspension fluid and tighten cap.
5. Place the blue cap on the uncapped culture vial and dispose of it in the biohazard trashcan.
6. Invert the vial and place it in the foam rack.
7. Place the foam rack with the three vials in the incubator for 10 minutes.
8. After 10 minutes remove vial from the incubator and tap on the cap gently to mix.
9. Remove the cap and examine the inside surface. If any un-dissolved black particles remain, return the cap to vial, invert and incubate for another 10 minutes.

Repeat steps 4-11 for the remaining cultures.

10. Remove labeled pre-warmed pre-filled bottles from the incubator and place within workspace.
11. Loosen but do not remove the lids.
12. Invert each re-suspended culture vial gently to mix and add the entire contents of the vial into the appropriate sample vessel.
13. Return the lid to the vial and dispose in the biohazard trashcan.

Once the vial contents have been added to each bottle the samples can be processed using the general Sample Processing Procedures with the exception that the bottles used must be discarded in the biohazard trashcan as they contained live bacterial cultures.

Disinfect all work surfaces when finished and remove gloves by rolling them inside out and discard them in the trashcan.

Recording:

Annotate the bacteria processing worksheet with the time and date the samples were placed in the incubator, the name of the processor, the date and time the samples were processed, the time the samples are to be removed from the incubator on the following day, and the incubator temperature prior to sample incubation.

After an incubation time of 18 hours, the Quantitrays should be removed from the incubator and processed using the general Sample Results Procedures. Annotate the bacteria processing worksheet with the time and date the samples were removed from the incubator, the name of the processor reading the trays and the incubator temperature prior to sample removal.

- Boxes of Colilert-18 media snap-packs that have met the QA/QC standard using the Quanti-Cult cultures should be labeled, in permanent marker, “QA/QC’d by:” printed in clear view followed by the processor’s initials and the date.
- If the media does not meet the QA/QC requirements outlined above, the box should be labeled “Failed QA/QC” and include the analyst’s initials and the date. Boxes of media that fail QA/QC should then be disposed in the trashcan.
- Annotate the Quanti-cult Quality Assurance Sheet with the appropriate information.

Table 6 Logbook Header – Quanticult for Colilert-18 Media

| Date | Lot Number | Yellow | | | | Yellow + Fluorescence | | | | Analyst | QA/QC Pass/Fail |
|------|------------|---------|---------------|---------------|-------|-----------------------|---------------|---------------|-------|---------|-----------------|
| | | E. Coli | K. pneumoniae | P. aeruginosa | Blank | E. Coli | K. pneumoniae | P. aeruginosa | Blank | | |
| | | +/+ | +/- | -/- | | +/+ | +/- | -/- | | | |

Incubator

Three types of incubators are used in this program. The primary unit used by the SJR Watershed Unit is Fisher Scientific Isotemp Forced Air Incubator, Model 650F. The second incubator is the Binder® Incubator used by the CVRWQCB-Sacramento office lab. The Type I42300 Incubator manufactured by Barnstead/Thermolyne Corp. is also included in our program for use of stakeholder groups where bringing samples to our office for processing is impractical due to hold time issues. The Colilert-18 Test Kit requires a temperature of 35°C +/- 0.5°C to meet bacteriological analysis requirements. The incubator temperature should be written on the bacteria processing worksheet **each time** samples are put in and again when samples are removed.

Initial Set up

- Chose a location that will provide an area that allows at least 2” of open space on all sides of the unit.
- Place the incubator in a convenient, level location.

- If using the Barnstead/Thermolyne Type I42300, ensure that the area is **FREE OF DRAFTS** and extraneous vibration.
- Leave incubator off (if using Fisher Scientific Isotemp or Binder Incubator) or unplugged (if using the Barnstead/Thermolyne I42300 incubator) when not in use.
- Ambient temperature must be at least 5-C below operating temperature. (Binder)

Fisher Scientific Isotemp Forced Air Incubator, Model 650F

Safety concerns

- **This unit is not explosion proof**, do not use in the presence of flammable or combustible materials; fire or explosion may result. Unit contains components, which may ignite such materials.
- Fumes and spillage from acidic solutions cause corrosion of the stainless steel chamber and other components. Care should be taken to maintain a neutral pH at all times.
- The heater is in the bottom of the unit. Surface temperatures at the bottom cover may be higher than set point temperature.
- A deviation alarm alerts the operator and interrupts heater power whenever the actual incubator temperature differs from the set temperature by more than 3-C. The operator cannot adjust this set limit.
 - If the actual temperature exceeds the alarm limit, the alarm indicator LED will light and the Display will flash “EEE”.
 - The reference point for the alarm is the set temperature.
 - Changing the set temperature to a value more than 3-C below the present incubator temperature will trip the alarm. Power is removed from the heater when an alarm condition occurs until the actual temperature is within 3-C of the set temperature, at which point the unit alarm LED will turn off and the display will resume normal operation.

Setting the temperature

1. In control mode operation, the incubator maintains a set temperature until that set temperature is changed. The Actual and Set Displays will indicate current chamber temperature and the temperature set point, respectively. To set a temperature and initiate Control mode operation, perform the following:
2. Place the power switch in the ON position. All 8's will flash as a test of the display
3. Press and hold the SET key.
4. Observe the set temperature in the Set Display window.
5. To decrease the set temperature, press the DECREASE Key while holding the SET key.

6. To increase the set temperature, press the INCREASE Key while holding the SET Key.
7. When the desired set temperature is shown, release the INCREASE OR DECREASE Key. Finally, release the SET Key. The incubator automatically begins to control at the set temperature
8. NOTE: Upon initial heat up to temperature, the over range alarm indicator light may illuminate briefly (several minutes) until the temperature stabilizes at the set point. The incubator temperature should be allowed to stabilize prior to loading samples.

Calibrating the offset of the Fisher Scientific Isotemp Forced Air Incubator, Model 650F

1. This incubator must be calibrated to ensure the digital readout offset is correct. (Allows the operator to measure and calibrate the digital display so that the display indicates the temperature at a specific point or zone within the incubator, rather than the temperature measured at the control thermocouple)
2. Place at least one thermometer in the incubator.
 - a. If one thermometer is being used, place it on the middle shelf.
 - b. If more thermometers are available, place one on each shelf.
3. Press the MENU key, the display will indicate CAL
4. To view the present offset value, press and hold the SET Key.
5. To change the display offset, press and hold the SET Key. Press the INCREASE arrow key or DECREASE arrow key until the display indicates the desired offset.
6. Release the SET Key.
7. Press MENU Key once to return to normal temperature control.

QA Specifications:

| | |
|---------------------------|------------|
| Average Uniformity @ 37-C | +/- 0.3 –C |
| Resolution | 0.1 –C |
| Control Sensitivity | +/- 0.1 –C |
| Recovery Time @ 37 –C | 3 minutes |

If the incubator is not working within these specifications, contact the program manager and advise of the concern. The program manager will inspect the incubator to determine if the malfunction can be identified and fixed or if the concern needs to be elevated to the manufacturer.

Troubleshooting

Table 7 Fisher Scientific Isotemp 650F Troubleshooting

| Symptom | Probable Cause | Action |
|----------------|----------------------------------|-------------------------|
| No Power | Unit not plugged in or turned on | Plug in or turn on |
| | Defective circuit | Replace circuit breaker |

| | | |
|---|--|---|
| | breaker | |
| | Wire disconnected | Replace loose wires |
| Incubator temperature erratically high | Defective control thermocouple | Replace control thermocouple |
| Failure to heat | Defective control board | Replace control board |
| | Set temperature less than actual temperature | Refer to <i>Operation</i> |
| | Defective control thermocouple | Replace control thermocouple |
| | Poor heater connections | Tighten connections at terminal strip and/or heater |
| | Defective heater element | Check heater resistance on schematic at back of manual. Replace heater unless approximately the same as schematic. |
| | Defective solid state relay or safety relay | Refer to schematic and replace relay. |
| | Defective controller | Replace controller |
| Alarm LED stays on and control is higher than set temperature | Set temperature has been changed to a value less than the actual temperature minus the alarm limit | Wait for actual temperature to cool to the set temperature. (To set plus 3 degrees and LED will go out and EEE display back to normal operation.) |
| | Defective controller | Replace controller |
| | Defective solid state relay or safety relay | Refer to schematic and replace relay. |
| Set Display Shows "EEE" | Alarm LED also lit | Allow incubator to cool to set point See above. |
| | Alarm LED not on; Faulty or broken connections | Check thermocouple connections at rear of temperature controller |

| | | |
|--|------------------------------------|--|
| | Defective control | Replace control if thermocouple is OK |
| Temperature off from Independent thermometer | Calibration offset needs adjusted. | Begin by setting offset to 0. (See Display Offsets.) |

- Service procedures involve exposure to line voltage and should be done only by qualified service personnel. Disconnect incubator from power source before attempting repairs. The procedures for replacing the Door Gasket, Replacing the door handle, and adjusting the door handle may be performed by most users. Details for these procedures and replacement part numbers can be found in the Fischer Isotemp Incubator User's Manual.
- For Technical Assistance, Call 1(800) 926-0505
- For Field Service Division Assistance, Call 1(800) 395-5442

Binder BD53 Incubator

Safety Concerns

- Keep safety labels complete and legible.
- The incubator must not be operated in hazardous locations.
- This incubator does not dispose of any measures of explosion protection.
- The unit must NOT become wet during operation or maintenance
- Do NOT touch the inner surfaces, the door window, the access ports, exhaust duct, and the charging material during operation, as they will become hot.
- Respecting the instructions in the operating manual and conducting regular maintenance work is part of the intended use.
- Do NOT use the unit for drying purpose, especially if greater quantities of steam leading to condensation will be set free.
- Do NOT lift or transport the unit using either the door handle or the door.
- Lifting the incubator requires two people.
- The ambient temperature should not be substantially higher than the indicated ambient temperature of +25 –C to which the specified technical data relate.
- Do NOT exceed the maximum temperature set point of 50 –C.
- Change the door gasket in cold condition only. Otherwise the door gasket will be damaged.
- Do NOT spill water or cleaning agents over the inner and outer surfaces.
- Put off-circuit the unit before cleaning. Pull the power plug.
- Completely dry the appliance before switching it on again. Do NOT use acidic or chlorine cleaning detergents
- Safety Device

Calibrating the thermostat

- The safety control thermostat must be calibrated once the incubator reaches the set point
 1. Turn the control knob of the safety device using a coin or a screwdriver to its end-stop (position 10).
 2. Turn the control knob back until its trip point - (turn it counter clockwise)
 3. The trip point is identifiable by the red alarm lamp lighting up
 4. The optimum setting of the safety device is obtained by turning the knob clockwise by around one graduation mark on the scale.
 5. If the safety device has assumed the regulation function, proceed as follows;
 - a. Disconnect the unit from the mains.
 - b. Have the cause of the fault examined and rectified by a technician
 - c. Restart the unit

Setting the temperature

1. Air change control
 - a. This is adjusted using the lever under the control buttons.
 - b. The air change adjusts the amount of fresh air entering the incubator.
 - c. This needs to be kept in the closed position since an open-air flap can negatively influence the spatial temperature accuracy.
2. Switch on the incubator – the green “Standby” LED illuminates. (To the left of the temperature display).
3. Press the power button (circle with a line pointing up)
 - a. The actual temperature value will be displayed.
 - b. If the display alternately shows the actual temperature value and “tOFF”, the incubator is in timer operation mode and will need to be set to continuous operation.
 - i. If the display only reads the temperature, go to “Controller setting”. If not follow the directions below:
 - ii. To switch back to continuous operation:
 1. Press the time management button (circle with one line pointing up and one pointing to the 3 o'clock position)
 2. Press the down arrow button for 2 seconds. The display should alternate between “t1” and “tInF”.
 3. Press the X/W button to return to normal display/actual value display.
 - c. Controller Setting
 - i. Press X/W button
 - ii. The display will alternately show “SP” and the previous temperature set-point.

- iii. With the up and down arrow buttons, enter a set-point of 34.5-C.
- iv. Wait 2 seconds until the entered temperature value is taken over (display will flash once)
- v. Press the X/W button to return to normal display.

Table 8 Fisher Scientific Isotemp 650F QA Specifications

| | |
|---|----------------|
| Temperature variation at 37 –C (without outer glass door) | 0.5 +/- –C |
| Temperature fluctuation at 37 –C | 0.1 </- +/- –C |
| Heating up Time to 37 –C (up to 98% of the set value) | 38 Minutes |
| Recovery time after door was opened for 30 seconds (up to 98% of the set value) | 5 Minutes |

If the incubator is not working within these specifications, contact the program manager and advise of the concern. The program manager will inspect the incubator to determine if the malfunction can be identified and fixed or if the concern needs to be elevated to the manufacturer.

Barnstead/Thermolyne Corp. Type I42300 Incubator:

Safety Concerns

- o **NOTE:** Leave unit disconnected when not in use.
- o **DANGER:** Do not use in the presence or flammable or combustible materials or explosive gases. Do not use in the presence of pressurized or sealed containers. Fire or explosion may result, causing death or severe injury.
- o **WARNING:** Do not heat any substance above a temperature that will cause it to emit toxic fumes – death or severe injury may result.

Setting the temperature

1. Make sure the power switch is in the OFF position
2. Plug the cord set into a grounded outlet that provides the electrical specifications listed on the unit’s nameplate.
3. Place grommet into the center of the thermometer holder. Install the thermometer, supplied with the unit, through the grommet. Adjust the immersion depth to 3 inches or 76 mm (measured from the bulb to the top of the grommet). Insert the assembly into the vent on top of the incubator.
4. The incubator requires approximately 90 minutes to warm to 35 °C.
5. Adjust the temperature knob according to the temperature of the thermometer.
6. Wait for temperature on the thermometer to stabilize.
7. Make further adjustments as necessary.

QA:

1. To determine the ability of the incubator to produce and maintain a constant temperature, check the temperature prior to placing samples in the incubator and again just before sample removal 18 hours later. The temperature should read 35°C +/- 0.5°C. **This should be performed each time the incubator is used.**
2. Record both temperatures on the Bacteria Processing Sheet under the Incubator Maintenance heading.
3. If temperatures are beyond the specified limits, samples must be moved to a properly functioning incubator. Samples that remain at improper temperatures longer than two hours must be discarded and not reported.
4. Unplug the incubator when finished.

Incubator QA Performed with each run

Materials:

Bacteria processing sheet
Incubator
Thermometer

Record the temperature on the thermometer (if using the Barnstead/Thermolyne Incubator) or on the digital display (if using the Binder or Fisher Scientific Incubators) at the start of sample incubation and when the samples are pulled from the incubator.

Cleaning

1. Always unplug unit prior to cleaning
2. During cleaning take precautions to prevent cleaning agents from contacting electrical components
3. Use a mild, non-abrasive cleaner (i.e. damp cloth and mild soap or alcohol based solution) to clean all normally accessible surfaces
4. Clean the shelves and interior with water and mild detergent solution only. Do not use abrasives, cleansers or scouring pads. Rinse and dry completely.
5. Make sure unit is dry before re-connecting to power source

Thermometer

For general purposes, thermometers are graduated in 0.5°C increments or less (11). The incubator thermometer should be checked against a NIST certified or NIST traceable thermometer within or near the temperature ranges of intended use on a **semi-annual basis** (11).

Materials:

2 50ml beakers
Tap water
NIST Certified or NIST Traceable Thermometer (See Lab Supervisor)
Incubator thermometer
Incubator
Tape

Procedure:

1. Plug in Incubator (approximate warm-up time: 90 minutes). Do not adjust the temperature setting knob.
2. Fill two 50 mL beakers about half way with tap water
3. Place the beakers side by side on the bottom shelf within the incubator.
4. Place the NIST thermometer in one beaker and lab incubator thermometer in the other and close the incubator door.
 - a. The water prevents erratic changes in the temperature and allows a more accurate reading.
5. Make certain that the door is completely closed.
6. Allow the thermometers to set until their temperatures stabilize.
7. Label a piece of tape with the date, NIST reading, Lab thermometer reading and NIST serial number and place it on the lab thermometer.
8. Record the date, time, NIST reading, Lab thermometer reading, NIST serial number and analyst on the Incubator/Thermometer Maintenance sheet found in the QC Log book.

Table 9 Logbook Header - Thermometer

| Date | Time | NIST Thermometer Reading | Incubator Thermometer Reading | NIST Serial Number | Analyst | Comments |
|------|------|--------------------------|-------------------------------|--------------------|---------|----------|
|------|------|--------------------------|-------------------------------|--------------------|---------|----------|

Sealer

The purpose is to ensure the trays are properly sealed and the sealer is functioning correctly. The Quanti-tray[®] Sealer by IDEXX automatically distributes the sample/substrate mixture into separate wells. If the sealer is malfunctioning, it will not completely seal the wells of the Quanti-tray, allowing leakage between wells. This may cause results to be inaccurate since Most Probable Number (MPN) reporting is quantitative. QA/QC should be performed on the sealer on a monthly basis to assess its effectiveness. If the sealer is not operating correctly, another sealer should be obtained. For IDEXX technical assistance call 1-800-321-0207 or 207-856-0496.

Materials:

Gloves
Lab Coat
1 100ml IDEXX bottle
1 Quanti-tray
100ml of tap water
1-2 drops of food coloring
Rubber insert
Sealer

Procedures:

For personal safety in the lab, gloves and a lab coat should be worn.

Tray Seal Check

1. Turn on Sealer (takes ~ 15 minutes to warm up)
2. Fill a 120 mL IDEXX bottle to the 100 mL line with tap water
3. Add 1-2 drops of food coloring
4. Swirl bottle until color is mixed
5. Pour into a Quanti-Tray and tap tray bottom to remove air bubbles
6. Place tray in rubber insert
7. Feed the tray and insert through the sealer
8. Examine the sealed Quanti-tray for evidence of leakage between wells.
 - a. If there is no leakage, the sealer is in proper working order.
 - b. If leakage has occurred, consult owner's manual for repair information and/or troubleshooting.
 - c. Label the back of the Quantitray "Sealer QA/QC", the date and the analyst's initials in permanent marker.
9. Record the date, time, analyst initials and whether there was dye between the wells of the tray on the Sealer Maintenance sheet. This can be found in the QC Log book.
10. Dispose of tray in trashcan.

Sealer Cleaning

1. Ensure power supply is off, sealer is unplugged, and unit has completely cooled down for at least 90 minutes. Remove input tray shelf. Loosen four quarter-turn fasteners and remove the access panel.
2. Check roller for yellow/caramel color deposits. If deposits are not present, replace access panel. If deposits are present, follow these steps:
 - a. Loosen hold-down screws, which secure the lower roller assembly to the bottom plate of the sealer.
 - b. Remove lower roller by lifting straight up and then out, to ensure roller clearance of locating pins on the bottom plate of the sealer.
 - i. Be careful not to touch the upper roller if it is hot.

- c. Use mild detergent, diluted bleach, or isopropyl alcohol to clean all accessible surfaces inside the sealer and the lower roller assembly.
 - i. Never use abrasive materials for cleaning
 - ii. Never use caustic cleaners
 - iii. Use alcohol only on cool sealer.
 - iv. For stubborn deposits, allow soak time for cleaner to work.
 - v. Do not disassemble lower roller assembly.
- d. Dry interior and roller assembly with paper towels or soft cloth.
- e. Reinstall bottom roller assembly on locating pins and tighten hold-down screws.
- f. Fasten access panel and reattach tray shelf.

Table 10 Logbook Header - Sealer

| Date | Check Seal Effectiveness | Replace Roller | Check Feed | Clean Sealer | Check Rubber Insert | Comments |
|------|--------------------------|----------------|------------|--------------|---------------------|----------|
| | | | | | | |

UV Lamp

The UV lamp used to view samples positive for *E. coli* is Model EA-160 Spectroline® 6-watt long wave ultraviolet lamp which produces a wavelength of 365 nm. Preventive maintenance steps come from Laboratory Quality Assurance Guidance for Colilert® Analysis under the Clean Rivers Program. UV protective goggles must be worn when using the lamp. The bulb should be replaced after approximately 6,000 hours or daily use for about 5 years if used a few hours a day continuous usage or when it fails to pass QA/QC.

Materials:

Gloves
 Lab Coat
 UV Goggles
 Comparator
 UV Light
 UV Viewing Cabinet

Test UV lamp for proper fluorescence **monthly** using a known *E. coli* positive MMO-MUG comparator.

Procedures:

1. Place the UV light on the UV viewing cabinet. This will automatically place the light at six inches above the trays, the height necessary for proper viewing of fluorescing wells.

- a. To turn on the UV light, press the red button so that it pops up and then press the white button.
 - b. Press down on the red button to turn the UV light off.
2. Place the comparator within the viewing cabinet
3. The comparator should fluoresce under the UV light
4. Record the date, whether the lamp causes fluorescence, and the analyst on the UV Lamp Maintenance sheet found in the QA/QC Log book located in the lab.
5. Record all maintenance and tube replacement on the UV Lamp Maintenance sheet. Additional sheets can be found at W:\nps\San Joaquin River\IBP\Bacteria\ QA_QC\Bacti Equipment.

Table 11 Logbook Header - UV Lamp

| Date | Check Lamp Fluorescence | Check Bulb | Replace Bulb | Analyst | Comments |
|------|-------------------------|------------|--------------|---------|----------|
|------|-------------------------|------------|--------------|---------|----------|

Information on quality assurance and procedures regarding data completeness, accuracy, and precision can be found in Appendix A: Quality Assurance Project Plan Intensive Basin Investigation, CVRWQCB, 2004.

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Appendix A: Sample Field and Bacteria Processing Worksheets

This Appendix provides examples of the following:

- Blank Field Sheet
 - Completed Set up Field Sheet
- Blank Bacteria Processing Worksheet
- Completed Set up Bacteria Processing Worksheet

Run:

(revised 12/12/07)

Sampler:
Project:

Date:
Vehicle #

YSI METER #

YSI

| Sample ID - Code | Site Code | Site Description | Sampling Type | Time | Temp (C) | Field SC | DO mg/L | pH | Dry | Lab SC | Pic # |
|------------------|------------|------------------|---------------|------|----------|----------|---------|----|-----|--------|-------|
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| BacLD 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | | G | | | | | | | | |
| Bac 541 | 541 | DUP # | A | | | | | | | | |
| BacLD 541 | 541 | Lab Dup# | A | | | | | | | | |
| Bac | PBS | Travel Blank | A | | | | | | | | |
| Bac | PBS | Lab Blank | A | | | | | | | | |

| Time | Calibrations | Initials | pH | pH MV | | | DO | | Conductivity | | Recal | Lab |
|------|---------------------|----------|---------|-------|-------|------|----------|-----------|--------------|------------|--------------|-----|
| | | | 7.0 | 4.0 | 7.0 | 10.0 | Charge | Gain | Sp. Cond | Cal Const. | Y/N | EC |
| | Beginning | | | | | | | | | | | |
| | End | | | | | | | | | | | |
| | ph slope 165-180 MV | | +/- 0.2 | +177 | +/-50 | -177 | 50 +/-25 | -0.7to1.4 | +/- 20 | | 5.0 +/- 0.45 | |

BAC- Bacteria

BacLD + BacLD = ONE P/250 mL, split in lab into TWO P/120 mL

NOTES: WEATHER CONDITIONS, WATER LEVELS

G- Grab Sample

A- Refer to LabQA.xls

Run: Ag/GBP-4

*Note YSI: #1=19869; #2=19859; #3=18243; #4=18471

SJRWU Monitoring Data Sheet
(revised 12/12/07)

Sampler: VAW
Project: Ag/GBP

Date: 12/16/2007
Vehicle # 1

YSI METER # 1

YSI

| Sample ID - Code | Site Code | Site Description | Sampling Type | Time | Temp (C) | Field SC | DO mg/L | pH | Dry | Lab SC | Pic # |
|------------------|------------|------------------|-------------------------------------|------|----------|----------|---------|----|-----|--------|-------|
| 24 | Bac | 541MER 522 | SJR @ Lander | G | | | | | | | |
| 10 | Bac | 541MER 531 | Salt Sough @ Lander | G | | | | | | | |
| 11 | Bac | 541MER535 | Dschrg from S L D (evidence) | G | | | | | | | |
| 12 | Bac | 541MER542 | Mud Slough downstream of SLD | G | | | | | | | |
| 13 | Bac | 541MER536 | Mud Slough Upstream of SLD | G | | | | | | | |
| 14 | Bac | 541MER538 | SJR @ Fremont Ford | G | | | | | | | |
| 15 | Bac | 535STC504 | SJR @ Crows Lndng (evidence) | G | | | | | | | |
| 16 | BacLD | 541STC507 | SJR @ Patterson | G | | | | | | | |
| 17 | Bac | 541STC510 | SJR @ Maze | G | | | | | | | |
| 18 | Bac | 541SJC501 | SJR @ Airport Way | G | | | | | | | |
| 21 | Bac | 541MER535 | DUP # 11 SLD | A | | | | | | | |
| 20 | BacLD | 541STC507 | Lab Dup# 16 SJR@Patterson | A | | | | | | | |
| 22 | Bac | PBS | Travel Blank | A | | | | | | | |
| 23 | Bac | PBS | Lab Blank | A | | | | | | | |

| Time | Calibrations | Initials | pH | pH MV | | | DO | | Conductivity | | Recal | Lab |
|------|---------------------|----------|---------|-------|-------|------|----------|-----------|--------------|--------------|-------|-----|
| | | | 7.0 | 4.0 | 7.0 | 10.0 | Charge | Gain | Sp. Cond | Cal Const. | Y/N | EC |
| | Beginning | | | | | | | | | | | |
| | End | | | | | | | | | | | |
| | ph slope 165-180 MV | | +/- 0.2 | +177 | +/-50 | -177 | 50 +/-25 | -0.7to1.4 | +/- 20 | 5.0 +/- 0.45 | | |

BAC- Bacteria

BacLD + BacLD = ONE P/250 mL, split in lab into TWO P/120 mL

NOTES: WEATHER CONDITIONS, WATER LEVELS

G- Grab Sample

A- Refer to LabQA.xls

| | | | | | | | | | | | | | | | |
|---|-------------------------|------------|--------|--------------|---|--------------|---------------------------|------------------------|--------------|-------------------------|-------------------------|------------|------------|------------|-----|
| Run: | | | | | | | | | | Sample Processing Date: | | | | | |
| Sample ID #: | | | | | | | | | | 12 | 14 | 16 | 18 | 21 | 23 |
| Site Code: | | | | | | | | | | MER 542 | MER 538 | STC 507 | SJC 501 | STC 507 | PBS |
| Yellow + | # Small Wells | | | | | | | | | | | | | | |
| | # Large Wells | | | | | | | | | | | | | | |
| | Empty Wells | | | | | | | | | | | | | | |
| | MPN | | | | | | | | | | | | | | |
| Yellow + Fluorescence (+) | # Small Wells | | | | | | | | | | | | | | |
| | # Large Wells | | | | | | | | | | | | | | |
| | False Positives | | | | | | | | | | | | | | |
| | MPN | | | | | | | | | | | | | | |
| Temp/Time | Start | 4Hr. Check | | | | 14 Hr. Check | | | 18 Hr. Check | | 22 Hr. Check, if needed | | | | |
| | FIELD DUPLICATES | | | | | | | LAB DUPLICATES | | | | | | | |
| | Normal Sample # | | | | | | | Normal Sample # | | | | | | | |
| Duplicate Sample # | | | | | | | Duplicate Sample # | | | | | | | | |
| | | MPN | 95% CI | | | | | | MPN | 95% CI | | | | | |
| | | | Lower | Upper | | | | | Lower | Upper | | | | | |
| TOTAL COLIFORM | Normal | | | | | | | Normal | | | | | | | |
| | Duplicate | | | | | | | Duplicate | | | | | | | |
| | Mean | | Pass | Needs Review | | | | Mean | | Pass | Needs Review | | | | |
| E. COLI | Normal | | | | | | | Normal | | | | | | | |
| | Duplicate | | | | | | | Duplicate | | | | | | | |
| | Mean | | Pass | Needs Review | | | | Mean | | Pass | Needs Review | | | | |
| BLANKS | Field Sample # | | Pass | Needs Review | | | | Lab Sample # | | Pass | Needs Review | | | | |
| Mean = Mean of Normal and Duplicate, which is then compared to the individual corresponding CI's to determine acceptability of data | | | | | | | | | | | | | | | |
| Sampler Signature / Date / Time Arrived: | | | | | Placed in Incubator By / Date / Time: | | | | | Trays Read By: | | | | | |
| Processor / Date / Time: | | | | | Pulled from Incubator By / Date / Time: | | | | | Entered into database: | | | | | |
| NOTES: | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | |
|---|-------------------------|------------|------------|---|--------------|------------|------------|-----------------------|----------------------------------|------------------------|-------------------------|--------------|-----|-----|------------|--|--|
| Run: Ag/GBP Run-4 | | | | | | | | | Sample Processing Date: 12/26/07 | | | | | | | | |
| Sample ID #: VAW071216- | | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 20 | 21 | 22 | 23 | 24 | | |
| Site Code: | | MER 531 | MER 535 | MER 542 | MER 536 | MER 538 | STC 504 | STC 507 | STC 510 | SJC 501 | STC 507 | MER 535 | PBS | PBS | MER 522 | | |
| Yellow + | # Small Wells | | | | | | | | | | | | | | | | |
| | # Large Wells | | | | | | | | | | | | | | | | |
| | Empty Wells | | | | | | | | | | | | | | | | |
| | MPN | | | | | | | | | | | | | | | | |
| Yellow + Fluorescence (+) | # Small Wells | | | | | | | | | | | | | | | | |
| | # Large Wells | | | | | | | | | | | | | | | | |
| | False Positives | | | | | | | | | | | | | | | | |
| | MPN | | | | | | | | | | | | | | | | |
| Temp/Time | Start | 4Hr. Check | | | 14 Hr. Check | | | 18 Hr. Check | | | 22 Hr. Check, if needed | | | | | | |
| TOTAL COLIFORM | FIELD DUPLICATES | | | | | | | LAB DUPLICATES | | | | | | | | | |
| | Normal Sample # | 16 | | | | | | | Normal Sample # | 11 | | | | | | | |
| | Duplicate Sample # | 20 | | | | | | | Duplicate Sample # | 21 | | | | | | | |
| | | MPN | 95% CI | | | | | | MPN | 95% CI | | | | | | | |
| | | Lower | Upper | | | | | | Lower | Upper | | | | | | | |
| | Normal | | | | | | | Normal | | | | | | | | | |
| | Duplicate | | | | | | | Duplicate | | | | | | | | | |
| | Mean | | | Pass | Needs Review | | | Mean | | | Pass | Needs Review | | | | | |
| E. COLI | Normal | | | | | | | Normal | | | | | | | | | |
| | Duplicate | | | | | | | Duplicate | | | | | | | | | |
| | Mean | | | Pass | Needs Review | | | Mean | | | Pass | Needs Review | | | | | |
| BLANKS | Travel Smpl # 22 | | | Pass | Needs Review | | | Lab Sample # 23: | | | Pass | Needs Review | | | | | |
| Mean = Mean of Normal and Duplicate, which is then compared to the individual corresponding CI's to determine acceptability of data | | | | | | | | | | | | | | | | | |
| Sampler Signature / Date / Time Arrived: | | | | Placed in Incubator By / Date / Time: | | | | | | Trays Read By: | | | | | | | |
| Processor / Date / Time: | | | | Pulled from Incubator By / Date / Time: | | | | | | Entered into database: | | | | | | | |
| NOTES: | | | | | | | | | | | | | | | | | |

Appendix B: Equipment Checklist

| Please check all boxes that apply | | | | | | | | | | | | | |
|---------------------------------------|--|--|--|--|--|--|--|--|--|----------|--|----------|--|
| | | | | | | | | | | Date | | Date | |
| | | | | | | | | | | Initials | | Initials | |
| Field Sheets | | | | | | | | | | | | | |
| Field - AG, SWAMP, IB | | | | | | | | | | | | | |
| Sigma - SLD/SLDB, CRW | | | | | | | | | | | | | |
| Clipboard | | | | | | | | | | | | | |
| Pencils, labels, photo sheets | | | | | | | | | | | | | |
| COCs | | | | | | | | | | | | | |
| CLS, Sierra, Twinning, Basic | | | | | | | | | | | | | |
| Field Binders | | | | | | | | | | | | | |
| Green AG - Weekly Runs | | | | | | | | | | | | | |
| Green AG & SWAMP - Monthly Runs | | | | | | | | | | | | | |
| Keys to access sites | | | | | | | | | | | | | |
| IB Binder - Weekly & Monthly IB Runs | | | | | | | | | | | | | |
| Camera | | | | | | | | | | | | | |
| Extra/charged batteries | | | | | | | | | | | | | |
| 3-1/2 disk - Cannon | | | | | | | | | | | | | |
| Phone | | | | | | | | | | | | | |
| Charged battery | | | | | | | | | | | | | |
| Maps | | | | | | | | | | | | | |
| Southern and Northern | | | | | | | | | | | | | |
| YSI | | | | | | | | | | | | | |
| Full service kit | | | | | | | | | | | | | |
| Screwdriver | | | | | | | | | | | | | |
| Charged battery | | | | | | | | | | | | | |
| Cleaned w/good DO membrane | | | | | | | | | | | | | |
| Truck | | | | | | | | | | | | | |
| Blue truck bag | | | | | | | | | | | | | |
| Keys, Credit card, Travel log | | | | | | | | | | | | | |
| Tire Inspection: Pressure/Tread | | | | | | | | | | | | | |
| Windshield Washer/ Oil/ Coolant | | | | | | | | | | | | | |
| Tire Kit: Full Spare/ Jack/ Tire Iron | | | | | | | | | | | | | |
| Bacteria | | | | | | | | | | | | | |
| Bacteria processing sheet | | | | | | | | | | | | | |
| Incubator turned on | | | | | | | | | | | | | |
| Bacteria cooler | | | | | | | | | | | | | |
| Ice pack | | | | | | | | | | | | | |
| Labeled bacteria bottles | | | | | | | | | | | | | |
| PBS Lab and Field blanks | | | | | | | | | | | | | |
| Reminder to pick up PBS Field blank | | | | | | | | | | | | | |
| Sigma Bases - AG runs | | | | | | | | | | | | | |
| Labeled Bottles | | | | | | | | | | | | | |
| DI Blanks | | | | | | | | | | | | | |
| G1 - SLD, CRW | | | | | | | | | | | | | |
| G1 EC - SLD, CRW | | | | | | | | | | | | | |
| Charged batter - SLDB, CRW | | | | | | | | | | | | | |
| AG/Swamp Coolers | | | | | | | | | | | | | |
| Labeled bottles | | | | | | | | | | | | | |
| DI Blanks - Monthly Runs | | | | | | | | | | | | | |
| Spare bottles | | | | | | | | | | | | | |
| Duplicate split bottles - A, B, S | | | | | | | | | | | | | |
| Bags of ice in ice machine | | | | | | | | | | | | | |
| Panache Cooler - AG runs | | | | | | | | | | | | | |
| 7 large sigma wedges | | | | | | | | | | | | | |
| 7 EC pints | | | | | | | | | | | | | |
| 2-COCs - _____ | | | | | | | | | | | | | |
| 2-Field sheets - SLDA, Panache | | | | | | | | | | | | | |
| 9-labels | | | | | | | | | | | | | |
| Blue ice pack | | | | | | | | | | | | | |
| pH Kit | | | | | | | | | | | | | |
| Blue Myron | | | | | | | | | | | | | |
| pH 4, 7, 10 | | | | | | | | | | | | | |
| EC 3900, 1417 | | | | | | | | | | | | | |
| DI, Tap H2O | | | | | | | | | | | | | |
| Alcohol spray bottle | | | | | | | | | | | | | |
| Soap | | | | | | | | | | | | | |
| Stainless steel & glass cups | | | | | | | | | | | | | |
| Gloves - Sm, Med, Lrd, X-Lrg | | | | | | | | | | | | | |
| Safety glasses, pens, paper towels | | | | | | | | | | | | | |
| EC Box | | | | | | | | | | | | | |
| Quarts, Pints, Bacteria bottles | | | | | | | | | | | | | |
| Garage Equipment | | | | | | | | | | | | | |
| Backup Sigma - Ag runs | | | | | | | | | | | | | |
| Tool Box | | | | | | | | | | | | | |
| Jumper Cables/ Flares (Red Box) | | | | | | | | | | | | | |
| First Aid Kit | | | | | | | | | | | | | |
| Bucket w/Rope & Insect Spray | | | | | | | | | | | | | |
| Extra bucket (for Tox) | | | | | | | | | | | | | |
| Shovel | | | | | | | | | | | | | |
| Potable Water | | | | | | | | | | | | | |
| Life Vests (1 for each sampler) | | | | | | | | | | | | | |
| Rubber Boots - <i>if needed</i> | | | | | | | | | | | | | |
| Rain Suits - <i>if needed</i> | | | | | | | | | | | | | |
| Sampling Poles | | | | | | | | | | | | | |
| Clamp - TOC, BAC | | | | | | | | | | | | | |
| Standard - pints, Quarts | | | | | | | | | | | | | |
| PVC - TOX | | | | | | | | | | | | | |
| Trach Bag & ER Bottles | | | | | | | | | | | | | |

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Comparison Counting Worksheet

(Updated 4 April 2008)

| Total Coliform | | | | | <i>E. coli</i> | | | | | | |
|----------------|---|---|---|-------------|----------------|---|---|----|-------------|---|---|
| Large Wells | | | | Small Wells | Large Wells | | | | Small Wells | | |
| 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 1 | | | | 1 | | | | 1 | | | |
| 2 | | | | 2 | | | | 2 | | | |
| 3 | | | | 3 | | | | 3 | | | |
| 4 | | | | 4 | | | | 4 | | | |
| 5 | | | | 5 | | | | 5 | | | |
| 6 | | | | 6 | | | | 6 | | | |
| 7 | | | | 7 | | | | 7 | | | |
| 8 | | | | 8 | | | | 8 | | | |
| 9 | | | | 9 | | | | 9 | | | |
| 10 | | | | 10 | | | | 10 | | | |
| 11 | | | | 11 | | | | 11 | | | |
| 12 | | | | 12 | | | | 12 | | | |
| 13 | | | | 13 | | | | 13 | | | |
| 14 | | | | 14 | | | | 14 | | | |
| 15 | | | | 15 | | | | 15 | | | |
| 16 | | | | 16 | | | | 16 | | | |
| 17 | | | | 17 | | | | 17 | | | |
| 18 | | | | 18 | | | | 18 | | | |
| 19 | | | | 19 | | | | 19 | | | |
| 20 | | | | 20 | | | | 20 | | | |
| 21 | | | | 21 | | | | 21 | | | |
| 22 | | | | 22 | | | | 22 | | | |
| 23 | | | | 23 | | | | 23 | | | |
| 24 | | | | 24 | | | | 24 | | | |
| 25 | | | | 25 | | | | 25 | | | |
| 26 | | | | 26 | | | | 26 | | | |
| 27 | | | | 27 | | | | 27 | | | |
| 28 | | | | 28 | | | | 28 | | | |
| 29 | | | | 29 | | | | 29 | | | |
| 30 | | | | 30 | | | | 30 | | | |
| 31 | | | | 31 | | | | 31 | | | |
| 32 | | | | 32 | | | | 32 | | | |
| 33 | | | | 33 | | | | 33 | | | |
| 34 | | | | 34 | | | | 34 | | | |
| 35 | | | | 35 | | | | 35 | | | |
| 36 | | | | 36 | | | | 36 | | | |
| 37 | | | | 37 | | | | 37 | | | |
| 38 | | | | 38 | | | | 38 | | | |
| 39 | | | | 39 | | | | 39 | | | |
| 40 | | | | 40 | | | | 40 | | | |
| 41 | | | | 41 | | | | 41 | | | |
| 42 | | | | 42 | | | | 42 | | | |
| 43 | | | | 43 | | | | 43 | | | |
| 44 | | | | 44 | | | | 44 | | | |
| 45 | | | | 45 | | | | 45 | | | |
| 46 | | | | 46 | | | | 46 | | | |
| 47 | | | | 47 | | | | 47 | | | |
| 48 | | | | 48 | | | | 48 | | | |
| 49 | | | | 49 | | | | 49 | | | |

Sample ID

Date of Comparison Count

Analyst ID

Time From Start of Incubation

Results:

| Total Coliform | | |
|----------------|-------------|-------------|
| | Large Wells | Small Wells |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| <i>E. coli</i> | | |
| | Large Wells | Small Wells |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| MPN Totals | | |
| Total Coliform | | |
| <i>E. coli</i> | | |

Comparison Counting Key:
1 = Dark Yellow/Dark Fluorescence
2 = Light Yellow/Light Fluorescence (Need Comparator to
3 = Colorless/No Fluorescence
4 = Colorless/No Fluorescence (Need Comparator to Determine)

Directions:

Complete the information for Sample ID, Analyst ID, and Time from start of incubation. Place the Quantitray-2000 numbered overlay over the tray to be used for Comparison Counting. Match the well to be analyzed with the well type and number identified on this worksheet. Using the Comparison Counting Key, place a "1" in the column that best fits your interpretation of the well. Once all wells have been analyzed for total coliform and *E. coli*, tally the number of each well fitting each description, and enter the result in the corresponding "Results" section. Analyst results should be