PURPOSE

To describe the procedure to monitor for viable airborne bacteria with the Remote-Slit-Sampler Confined Space Assembly (RCG) from EMTEK, LLC.

Principle

The RCG Confined Space sampling head is attached to a critical zone, or location using a length of sample tubing with sanitary connectors, or barbed fittings. A 100 mm agar based test plate is placed on the turntable of the RCG underneath the dome assembly and the dome assembly retention spring/tube is put in place. The required sample time period is set on the R2SC (Sampler Controller) timer and the testing period is initiated. During testing the vacuum pump of the R2SC pulls 28.3. LPM (from the critical zone) Per Hour (LPM) into the RCG dome assembly through the sample chamber and sample slit. Airborne bacteria in the sampled volume of air become impinged (captured) on the test plate at this point. The sampled air volume is then drawn through the vacuum line into the vacuum inlet of the R2SC and then through the mass flow controlled system. The sampled air volume passes through the vacuum pump and is then exhausted from the R2SC, located outside of (or away from) the critical environment (or test area).

During testing, the test plate rotates on the turntable at 1 revolution per hour. This rotation removes recovered organisms from the direct path of incoming air to help prevent their desiccation, allows for easier enumeration of isolates recovered and allows for the determination of the time of their recovery. Upon completion of the test period, the test plate is removed from the RCG and is incubated for a designated time period at a specified temperature. Following the required incubation period, the number of bacterial Colony Forming Units (CFU) are enumerated and the sampled volume of the air is determined (e.g., 28.3 LPM multiplied by the sample time in minutes). The density of air borne bacteria per volume of air/gas tested can then determined

SCOPE

This SOP covers the following:

1. Sanitization
2. RCG Confined Space Sampling Assembly Set-Up and Testing
3. Storage and Transport
4. Sample Submission, Results Reading and Recording

RESPONSIBILITIES

It is the responsibility of all personnel performing air viable monitoring testing with the RCG Confined Space Sampling Assembly to be trained and proficient with this procedure.

REFERENCES

Calibration of the EMTechnologies RCG Sampling Assembly

Materials

- EM Technologies RCG Confined Space Sampling Assembly Calibrated for operation at 28.3 LPM
- Sanitary Sample Tubing Assembly and sanitary fitting clamps (single, or multiple) Autoclaved, or Chemically Sanitized for use.
- Standard 100 mm TSA Agar Test Plates
- 30-35°C and/or 20-25°C Incubators
- Gloves (Sterile or Clean)
- Low Particulate Shedding Wipes (e.g., Wipe All, Gamma Wipes or equivalent or better)
- Primary Disinfectants (e.g., Quaternary Ammonia: Steris TBQ, NPD)

Note: Phenolic disinfectants should not be used on the Clear Dome Assembly or White Transport Cup as they will cause them to become very brittle in a short period of use.

- Secondary Disinfectants (e.g., 70% 0.2µm Filtered or sterilized IPA)
- Lab Marker and/or Pre-Printed Labels
- Adhesive Tape

Maintenance Inspection

To assure appropriate operation of the RCG sampling assembly, prior to each days use, sampling personnel should inspect the unit for any obvious physical defect. This inspection shall include but not be limited to the following:

- A visual check of the dome-to-base seal or dome assembly to assure it is not visibly damaged in a way that would keep it from sealing appropriately (tears in the seal, cracks or chips in the dome, etc.).

- The sample slit of the sample throat shall be visually inspected to assure that it is free of occlusions to assure proper sample flow through the slit.

If any maintenance need be performed, contact appropriate service or repair personnel.

IMPORTANT SAFETY PRECAUTIONS!!

See RCG/R2SC Maintenance/Specifications Manual additional information prior to operation.

- TO MINIMIZE THE CHANCE OF ELECTRICAL HAZARD, assure that the primary AC power supply cord is not plugged in during sanitization.

1 GFCI PROTECTION

Assure the GFCI (Ground Fault Current Interruption) Protection Device is operating properly prior to each use. See Step 2.5.2.

WARNING

1. If the GFCI fails to trip when the Test Button is pressed, or fails to reset, the device is defective. Contact EMTechologies for warranty repair or replacement.

2. If the GFCI trips each time the cord is plugged in, then the controller or sampling head has a ground fault and needs to be repaired or replaced.

3. The GFCI does not sense ground faults in the input conductors. Therefore, when extension cords are used the GFCI provides not protection between itself and the AC outlet receptacle.

DO NOT BYPASS THE GFCI IF THIS CONDITION OCCURS. A REAL SHOCK HAZARD MAY EXIST.

2 DO NOT REMOVE THE PANELS or COVERS of the R2SC or RCG to attempt any repairs. Contact EM Technologies or other qualified service personnel if the unit malfunctions.

- DO NOT SUBMERSE the RCG or R2SC in any liquids!

- TAKE ALL OTHER STANDARD ELECTRICAL SAFETY PRECAUTIONS when operating the RCG Sampling Assembly

PROCEDURE

1. Sanitization

1.1 Don a pair of clean or sterile gloves

NOTE: Gloved hands should be cleaned with secondary disinfectant (i.e., 70% IPA) throughout this procedure.

CAUTION: TO MINIMIZE THE CHANCE OF ELECTRICAL HAZARD, assure that the R2SC primary AC power supply cord is not plugged in during sanitization.

DO NOT SUBMERSE the RCG or R2SC in any liquids!

1.2 Upon bringing the RCG Controller unit into a critical area (e.g., uncontrolled to Class 100,000, 100,000 to Class 100, etc.), sanitize the exterior of the vacuum tubing, power supply cable, R2SC and primary AC power supply cord with a wipe saturated with disinfectant.

1.3 RCG Sanitization:

1.3.1 Remove the dome retention spring/tube from the dome assembly and remove the dome .

1.3.2 Sanitize the exterior surfaces of the RCG (i.e., the body, “dome-to-base seal”, turntable, turntable adjustment knob, etc.) with a new wipe saturated with secondary disinfectant.

NOTES:

- Be careful not to saturate the air passageway opening located beneath the turntable, as this would allow the disinfectant to be drawn into the vacuum pump and damage it.
- For initial cleaning each testing day, the dome-to-base seal may be removed for sanitization. Sanitize the surfaces of the seal with a wipe saturated with secondary disinfectant.

1.3.4 Dome Assembly Sanitization:

1.3.4.1 The dome assembly may be autoclaved prior to each days Testing if desired, or chemical sanitization may be found to be just as adequate.

1.3.4.2 For chemical sanitization of the dome assembly, saturate (spray/wipe, or submerge) the dome and sample chamber assembly with a primary disinfectant (i.e., TBQ or NPD), allowing appropriate contact time. Pour off and then wipe of any residual disinfectant with a new wipe.

1.3.4.3 Next, saturate the dome assembly with a sterile secondary disinfectant (i.e., 70% IPA, or EtOH). Allow for appropriate contact time and then pour off and wipe off any residual disinfectant with a new wipe.

1.3.5 Following sanitization, replace the dome-to-base seal on the RCG (if applicable), and then place the dome and sample chamber assembly back on the RCG. Assure that the dome is properly seated in the seal and the spring/tube is place.

1.3.6 The sanitary sample tubing assembly may be autoclaved before use each day if desired. Additional sets of sanitary sample tubing may be maintained (e.g., kept autoclaved) to assure a representative sample is taken from the desired sample points.

2. RCG Sampling Assembly Set-Up and Testing

2.1 Attach the RCG Power Supply Cable:

2.1.1 Attach the appropriate end of the power supply cable to the receptacle on the RCG. Firmly press the cable end connector onto the RCG connector while threading the collar clockwise until it becomes finger tight.

2.1.2 Attach the other end of the power supply cable to the receptacle on the R2SC. Firmly press the cable end connector in to the front panel connector while threading the collar clockwise until it becomes finger tight.

2.2 Place the RCG near the test site (e.g., on a bench, cart, table, etc.) within the constraints of the vacuum/power cable assembly and sanitary sample tubing (e.g., 3 feet)

2.3 Attach one end of the sanitized, or autoclaved, sanitary sample tubing/seal to the test site using a sanitary clamp assembly, barbed fitting, or other appropriate connector. Attach the other end of the sample tubing assembly to the sanitary fitting on the RCG sample inlet using a sanitary clamp.

Note: The dome assembly retention spring/tube is intended to retain the dome in place during set up, purging, and sampling.

2.5 Attach the RCG Vacuum Supply Tubing:

2.5.1 Attach one end of the vacuum tubing to the barb on the R2SC.

2.5.2 Attach the other end of the vacuum tubing to the barb on the RCG Sampler.

2.6 Initiate unit power as follows:

2.6.1 Plug the Primary AC Power Supply Cord with Ground Fault Current Interrupter into an appropriate 100-115 Volt / 60 Hz power outlet.

2.6.2 Perform GFCI Function Test per operations manual or as described on the GFCI.

2.6.3 Turn on the unit power switch found at the bottom back corner of the unit. This will initiate the unit's cooling fan and will illuminate the timer display.

2.7 Set the sample timer on the unit, using the up and/or down arrows, to the desired sample period (from 01" to 59' 59").

2.7.1 Holding the up or down keypads down for an extended period of time will accelerate the time setting on the unit.

2.7.2 To retain the sample period set, press the Start/Stop keypad on the timer before turning the unit power switch off.

2.7.3 To reset to the preset time, hold down the start/stop keypad until the time resets.

2.8 Setting the flow rate to 28.3 LPM:

2.8.1 Press the Start/Stop keypad on the timer to start the RCG vacuum pump.

2.8.2 Adjust the flow rate using the unit's rotometer (airflow controller and gauge). To achieve an airflow of 28.3 Standard Cubic Feet per Hour (LPM) through the sample throat and slit, adjust the rotometer controller knob so the flow indicator ball is centered at the specified setting on the rotometer set point indicator for 28.3 LPM.

2.8.3 Press the Start/Stop keypad on the timer to stop the vacuum pump.

2.9 Remove the retention spring/tube from the top of the dome assembly and place it on the edge of the dome seal.

2.10 Aseptically place the test plate (i.e., 100 mm TSA plate) on the turntable as follows:

NOTE: Gloved hands should be cleaned with secondary disinfectant immediately prior to performing these steps.

2.10.1 Document the following applicable information:

- Sample Start Time
- Initials/Date of Operator performing testing
- Sample Site Number or Description
- Calibration or Equipment Control Number of the RCG Sampling Assembly

2.10.2 Lift up the dome assembly just high enough to place the test plate on the turntable and remove its lid. Without inverting, place the lid of the test plate face down on a pre-sanitized surface next to the RCG.

NOTE: Removing and holding the dome only a few inches directly over the turntable will minimize the chance of contaminants settling on the sanitized components under the dome and on the test plate during this manipulation.

2.10.3 Place the dome assembly down over the test plate and turntable.

2.10.4 Securely seat the dome assembly in the “dome-to-base” seal and assure that the dome is properly seated in the seal.

2.10.5 Replace the dome assembly retention spring/tube.

2.11 Adjust the Turntable / Test Plate Height Adjustment as follows:

2.11.1 View the test plate distance indicator through the side of the dome to assure that test plate is at the proper height.

2.11.2 Rotate the turntable adjustment knob clockwise or counter clockwise until the top of the test plate moves the distance indicator pin into the position where the red anodized portions of the distance pin are no longer viewable (blocked from view by the distance pin mount). This adjustment will assure the required distance of approximately 2-3 mm from the test plate surface to the opening of the sample slit.

2.12 Starting the Testing Period:

2.12.1 Assure that the timer is set to the desired sample period and then press the Start/Stop keypad to begin the test cycle. Assure that the rotometer adjustment is at the indicated setting for 28.3 LPM.

2.13 Ending the Testing Period:

2.13.1 When the timer counts down from the set time period the vacuum pump will be automatically shut off. If desired, the test period can be terminated by pressing the Start/Stop keypad on the timer.

2.13.2 Remove the dome assembly retention spring/tube and place on the edge of the blue dome seal.

2.14 Aseptically remove the test plate from the turntable as follows:

NOTE: Gloved hands should be rinsed with secondary disinfectant immediately prior to performing these steps.

2.14.1 Remove the dome from the RCG with one hand and with the other hand; replace the lid of the test plate, being careful not to touch the inside of the lid or the agar surface. Do not move your hand over the exposed surface of the test plate. Allow the lid to lead your hand over the test plate as you replace it.

2.14.2 Remove the test plate, with the lid in place, from the turntable and place the dome back on the RCG and replace the dome retention spring/tube.

2.14.3 Secure the lid to the test plate with tape and document the “Sample Stop Time.”

2.14.4 Carefully inspect the test plate:

2.14.4.1 Without taking the lid off the test plate, assure that impingement marks are present on the agar surface and that they are reflective of the sampling period.

EXAMPLE:

The plate should make one full revolution if the timer is set for 59 minutes 59 seconds, and air impingement marks should be present around 360° of the plate. If the test period was 30-minutes, the impingement marks should be visible around 180° of the plate.

2.14.4.2 If impingement marks are not present, the sample/ test should be considered invalid. The sample should be retaken if possible.

2.14.5 Assure all applicable information is recorded on the test plate (i.e., Sample Start/Stop Time, Site #, Date, Operator Initials, Product information, etc.).

2.15 Repeat the applicable portions of this procedure for additional samples. A new sanitized sanitary sample tubing assembly may be used for each test site, or sample if desired. Chemical sanitization, or at least purging of the dome assembly should be performed at each sample location to assure a representative sample from that location following each set up.

3. Storage and Transport

3.1 For transport convenience:

3.1.1 The RCG should be placed in the transport mount located at the top of the R2SC (controller) for Transport (and for storage).

3.1.2 The RCG vacuum and power supply assembly should be coiled and placed over the RCG in the transport mount.

3.1.3 The Primary AC power supply cord and GFCI should be wrapped securely on the supplied cord wraps brackets on the back of the unit.

3.2 For transport outside of the facilities the entire assembly may be placed in a bag to minimize possible contamination of the unit.

3.3 Store the RCG Sampling Assembly in a clean and dry place.

3.4 A cover (i.e., sanitary cap, or bioshield) may be placed over the sanitary fitting opening of the sample conduit of the RCG during transport and storage to minimize contamination.

3.5 The sanitary sample tubing assembly may be autoclaved, or chemically sanitized before, or after each use. Although it is ideal to package and store the assembly as to minimize the contaminant load. Autoclave pouches may be used for this purpose.

4. Sample Submission, Results Reading and Recording

4.1 Submit the test plate(s) and test parameter information (i.e., date sampled, sampled by, start time, stop time, etc.) to QC Microbiology (*or other appropriate lab*) for log in. Qualified QC personnel shall record the required test parameter information on the appropriate electronic (e.g., LIMS) or hard copy test report form and place the test plates “On-Test” in incubation.

4.2 Incubate the test plates at 30-35°C for a minimum of 2-days, and transfer to 20-25°C for a minimum of 5-days (*other incubation requirements or schedules may be used*).

4.3 Following the required incubation period, count all Colony Forming Units (CFU) found within the air impingement zone. Differentiate between mold and bacteria when possible and record the results in the appropriate section of the test report form.

4.4 Determine Total CFU/ft3 and record the results in the appropriate section of the test report form. This result is calculated as follows:

$$\frac{\text{Total CFU per Test Plate}}{\text{[Time exposed (in minutes)/Plate] [Sample Rate (ft}^3\text{ / minute)]}} = \text{Total CFU/ft3}$$

EXAMPLE:

If the test period was 30-minutes and following incubation 60 CFU were recovered in the impingement zone of the plate:

- Total CFU/Test Plate = 60
- Time exposed in minutes = 30
- Sample Rate = 28.3 LPM = 1 ft3 / minute

$$\frac{\text{60 CFU/Test Plate}}{\text{[30 min./Test Plate] [1 ft3/min.]}} = \frac{\text{28.3 CFU}}{\text{30 ft 3}} = \text{2 CFU/ft3}$$

4.5 Record date “Off-Test” in the appropriate section of the test report form.

4.6 If Microbial Identification of organism recovered is required, submit plate for ID according to applicable procedures related for sample submission and processing for Microbial Identification.

4.7 Discard all other plates in an appropriate Bio-Hazard container for disposal.

4.8 Test Report Forms, once completed, shall be reviewed for accuracy and completeness, and signed by a second qualified analyst.