PURPOSE

To describe the procedure to monitor for viable airborne bacteria with the Remote-Autoclavable-Sampler Assembly (RAS) from EMTEK, LLC.

Principle

A 100 mm agar based test plate is placed on the turntable of the RAS underneath the dome assembly. The required sample time period is set on the R2S-C (Sampler Controller) timer and the testing period is initiated. During testing, the vacuum pump, of the R2S-C, pulls either 28.3 or 50 SPLM into the RAS assembly through the sample inlets. Air borne 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 R2S-C and then through the rotometer where the airflow is regulated. The sampled air volume passes through the vacuum pump and is then exhausted from the R2S-C, located outside of (or away from) the critical environment (or test area).

Upon completion of the test period, the test plate is removed from the RAS 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 air is determined (e.g., $1ft^3$ (28.3 Liters) per minute multiplied by the sample time in minutes). The density of air borne bacteria per volume of air tested can then determined

SCOPE

This SOP covers the following:

1. Sanitization
2. RAS 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 RAS Sampling Assembly to be trained and proficient with this procedure.

REFERENCES

Calibration of the EMTEK, LLC RAS Sampling Assembly

Materials

- EM Technologies RAS Sampling Assembly (RAS.PAK), Calibrated for operation at 28.3 or 50 Liters per minute
- 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)
- 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 RAS 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 base seal to assure it is not visibly damaged in a way that would keep it from sealing appropriately (tears in the seal, etc.).
- The sample inlets shall be visually inspected to assure that they are free of occlusions to assure proper sample flow.

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

IMPORTANT SAFETY PRECAUTIONS!!

See RAS/R2S-C 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.

- 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 EMTEK, LLC for warranty repair or replacement.

2. If the GFCI trips each time the cord is plugged in, then the controller 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.

- DO NOT REMOVE THE PANELS or COVERS of the R2S-C to attempt any repairs. Contact EMTEK, LLC or other qualified service personnel if the unit malfunctions.

- DO NOT SUBMERSE the R2S-C in any liquids!

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

PROCEDURE

1. Sanitization

1.1 Don a pair of clean or sterile gloves

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

CAUTION: TO MINIMIZE THE CHANCE OF ELECTRICAL HAZARD, assure that the R2S-C primary AC power supply cord is not plugged in during sanitization.

DO NOT SUBMERSE the R2S-C in any liquids!

1.2 Upon bringing the RAS Controller unit into a critical area (e.g., uncontrolled to ISO 8, ISO 8 to ISO 5, etc.), sanitize the exterior of the vacuum tubing, the R2S-C and primary AC power supply cord with a wipe saturated with disinfectant.

1.3 RAS Sanitization:

1.3.1 Sanitize the exterior surfaces of the RAS with a new wipe saturated with secondary disinfectant.

1.3.2 Remove the top sampling cover from the RAS.

1.3.4 With a new wipe saturated with secondary disinfectant, sanitize the interior of the RAS.

NOTES:

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

1.3.5 Following sanitization, replace the top sampling cover. Assure that the top sampling cover is properly seated on the seal.

NOTE: The entire RAS and seal assembly may be autoclaved.

2. RAS Sampling Assembly Set-Up and Testing

2.1 Attach the RAS Vacuum Supply Tubing:

2.2.1 Attach one end of the vacuum tubing to the barb on the R2S-C.

2.2.2 Attach the other end of the vacuum tubing to the barb on the RAS Sampler.

2.3 Upon completion of sanitization and set up (If applicable), place the RAS at the desired test site.

2.4 Place the R2S-C outside or below (i.e., on a cart) the testing area, within the constraints of the power supply cord and vacuum supply line.

NOTE: Contact EMTEK, LLC for additional information on the maximum length of the vacuum supply line allowable.

2.5 Initiate unit power as follows:

2.5.1 Plug the Primary AC Power Supply Cord with Ground Fault Current Interrupter into an appropriate 100-120 Volt / 60 Hz power outlet.

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

2.5.3 Turn on the unit power switch found at the bottom back corner of the R2S-C controller unit. This will initiate the unit's cooling fan and will illuminate the timer display.

2.6 Purge the unit prior to testing:

2.6.1 Start the vacuum pump by pressing the Start/Stop keypad on the timer. Run the vacuum pump for a period of time, roughly 30 seconds to 1 minute, to purge any residual moisture and particulate matter remaining from the sanitization.

2.6.2 Once the RAS unit has been sufficiently purged, press the Start/Stop keypad on the timer to stop the vacuum pump.

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:

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

2.8.2 Adjust the flow rate using the unit's rotometer (airflow controller and gauge). To achieve the desired airflow, adjust the rotometer controller knob so the flow indicator ball is centered at the specified setting (red indicator line) on the rotometer. This set point is determined through the calibration process and is set according to the desire flow rate (28.3 or 50 SLPM).

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

2.9 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 and allowed to dry immediately prior to performing these steps.

2.9.1 Document the following information (as applicable):

- Sample Start Time
- Initials/Date of Operator performing testing
- Sample Site Number or Description
- Calibration or Equipment Control Number of the RAS Sampling Assembly
- Product Description, Lot Number and Step Number

2.9.2 Lift up the top sampling cover 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 RAS.

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

2.9.3 Place the top sampling cover down over the test plate and onto the seal on the base plate holder.

2.9.4 Assure that the top sampling cover is properly seated on the seal.

2.11 Starting the Testing Period:

2.11.1 Assure that the timer is set to the desired sample period then press the Start/Stop key pad to begin the test cycle.

2.12 Ending the Testing Period:

2.12.1 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 Aseptically remove the test plate as follows:

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

2.13.1 Remove the top sampling cover with one hand and, with the other hand; replace the lid of the test plate Be 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.

2.13.2 Remove the test plate, with the lid in place, from the base plate holder and replace the top sampling cover.

2.13.3 Secure the lid to the test plate with tape and document the "Sample Stop Time" (if required).

2.13.4 Carefully inspect the test plate:

2.13.4.1 Without taking the lid off the test plate, assure that impingement marks are present on the agar surface.

2.13.4.2 If impingement marks are not present, the sample/test may be considered invalid.

NOTE: Due to excessive moisture of a test plate, impingement marks, may be "erased" from the agar surface. Ensure that plates with excessive moisture are not used to avoid this situation.

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

2.14 If additional samples are to be taken at the same location during the same test period (i.e., for continuous process monitoring), repeat Steps 2.9 through 2.13.5 for each additional sample required.

2.15 When testing is complete at a specified location:

2.15.1 Turn off unit power and disconnect from AC power supply.

2.15.2 Sanitize the RAS sampling assembly to remove any contaminants or test media.

2.16 If additional samples are to be taken at different locations in the same area (i.e., Fermentation) on the same day, move the RAS Sampling Assembly to the next location and repeat applicable portions of Steps 2.5 through 2.13.5. If the unit is to be used in a different area on the same day (i.e., moved from Fermentation to Purification) repeat applicable sanitization procedures in the next area prior to monitoring.

3. Storage and Transport

3.1 For transport convenience:

3.1.1 The RAS vacuum supply tubing should be coiled.

3.1.2 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 or storage outside of the production facilities, the entire assembly may be placed in a bag to minimize possible contamination of the unit.

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

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 the appropriate lab/department for log in. Qualified personnel shall record the required test parameter information on the appropriate electronic (e.x., LIMS) or hard copy test report form and place the test plates "On-Test" in incubation.

4.2 Incubate the test plates (inverted- agar side up) at 30-35°C for a minimum of 3-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/Liter and record the results in the appropriate section of the test report form. This result is calculated as follows:

$$\frac{\text{Total CFU per Test Plate}}{\text{[Time exposed (in minutes) for the Plate] [Sample Rate (Liters / minute)]}} = \text{Total CFU/Liter}$$

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 SLPM

$$\frac{\text{60 CFU/Test Plate}}{\text{[30 min./Test Plate] [28.3 SLPM]}} = \frac{\text{60 CFU}}{\text{849 (SLPM)}} = \text{0.07 CFU/Liter}$$

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.

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.