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.PURPOSE

To describe the procedure to monitor for viable airborne bacteria with the R2S Remote Start Sampling Assembly from EMT.

Principle

A 100 mm agar based test plate is placed on the turntable of the R2S 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 60 Standard Cubic Feet of air Per Hour (SCFH) into the R2S dome assembly through the sample throat and sample slit. 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).

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 R2S 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., 1ft³ 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. R2S Remote Start Sampling Assembly Set-Up and Testing
3. Storage and Transport
4. Sample Submission, Results Reading and Recording

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RESPONSIBILITIES

It is the responsibility of all personnel performing air viable monitoring testing with the R2S Sampling Assembly to be trained and proficient with this procedure.

REFERENCES

Calibration of the EM Technologies R2S Sampling Assembly

MATERIALS

- EM Technologies R2S Remote Start Sampling Assembly (R2S.RMTPAK), Calibrated for operation at 60 SCFH
- Standard 100 mm TSA Agar Test Plates (e.g., Becton Dickinson (Sterile Double Bagged), or equivalent or better
- 30-35°C Incubator(s) (or as appropriate per customer incubation scheme)
- Gloves (Sterile or Clean)
- Low Particulate Shedding Wipes (e.g., Wipe All, Gamma Wipes or equivalent or better)
- Primary Disinfectant (e.g, Steris NPD, TBQ)
- Secondary Disinfectant (e.g., 70% 0.2µm Filtered or sterilized IPA)
- Lab Marker and/or Pre-Printed Labels
- Adhesive Tape

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Maintenance Inspection

To assure appropriate operation of the R2S 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 R2S/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 operation properly prior to each use. See instructions on unit GFI, and/or Step 2.5.2 of this procedure.

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.

- DO NOT REMOVE THE PANELS or COVERS of the R2S-C or R2S to attempt any repairs. Contact EM Technologies or other qualified service personnel if the unit malfunctions.
- DO NOT SUBMERSE the R2S, R2S-C, Remote-Start-Switch, or any of the cabling assemblies in any liquids!

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- TAKE ALL OTHER STANDARD ELECTRICAL SAFETY PRECAUTIONS when operating the R2S Sampling Assembly

PROCEDURE

1. Sanitization: Upon bringing the R2S unit into a controlled environment for use (Transferring from uncontrolled to Class 100,000; 100,000 to Class 1,000 or 100, etc.), the following sanitization steps should be followed:
 - 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 R2S-C primary AC power supply cord is not plugged in during sanitization.

DO NOT SUBMERSE the R2S, R2S-C, Remote-Start-Switch, or cabling assemblies in any liquids!
 - 1.2 Sanitize the R2S Controller, exterior of the vacuum tubing, power supply cable, Remote-Start-Switch, and primary AC power supply cord with a wipe saturated with primary disinfectant. Then wipe the described components down with a wipe saturated with secondary disinfectant to remove residues.
 - 1.3 Sanitize the R2S Sampler Head as follows:
 - 1.3.1 Remove the dome assembly from the R2S.
 - 1.3.2 Sanitize the exterior surfaces of the R2S (i.e., the body, "dome-to-base seal", turntable, turntable adjustment knob, etc.) with a new wipe saturated with primary 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 potentially 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 primary disinfectant.
 - 1.3.3 With a new wipe saturated with primary disinfectant, sanitize the interior and the exterior of the dome and sample conduit assembly of the R2S.
 - 1.3.4 With a new wipe saturated with secondary disinfectants, wipe down all the R2S Sampler head components described.

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- 1.3.5 Following sanitization, replace the dome-to-base seal on the R2S (if applicable), then place the dome assembly back on the R2S. Assure that the dome is properly seated in the seal. Press the dome down firmly and rotate slightly to achieve a good seal.

NOTE: The dome assembly and dome-to-base seal may be autoclaved.

2. R2S Sampling Assembly Set-Up and Testing

2.1 Attach the R2S Power Supply Cable (If Applicable):

- 2.1.1 Attach the “female” end of the 3-Pin power supply cable to the “male” 3-Pin connector on the R2S Sampling Head. Firmly press the connector onto the receptacle while rotating the connector clockwise to thread it onto the connector.
- 2.1.2 Attach the “male” end of the 3-pin power supply cable to the “female” 3-pin connector on the Remote-Start-Switch assembly. Firmly press the connector on to the receptacle while rotating the end connector clockwise to thread it onto the connector.
- 2.1.3 Attach the “female” end of the 5-Pin power supply cable to the “male” 5-Pin connector on the Remote-Start-Switch. Firmly press the connector onto the receptacle while rotating the connector clockwise to thread it onto the connector.
- 2.1.3 Attach the “male” end of the 5-pin power supply cable to the “female” 5-pin connector on the front panel of the R2S Controller. Firmly press the connector on to the receptacle while rotating the end connector clockwise to thread it onto the connector.

Note: Alternate cabling assemblies may apply to your specific application. The same guidance generally applies, although additional connections may be required between the controller and the Remote-Start-Switch. All cabling and connectors up to the Remote-Start-Switch require 5-Pins and 5-Wire Leads.

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- 2.2 Attach the R2S 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 R2S Sampler.

Note: Alternate vacuum tubing assemblies may apply to your specific application (e.g., transfer panels). If in-line tubing connections are to be made, assure that the internal diameter (ID) of the tube adapters, or connectors are not so restrictive as to minimize the airflow through the tubing. Restrictive fittings in-line between the controller unit (vacuum pump) and R2S Sampling Head may cause a significant reduction in available vacuum at the R2S Sampling Head.
- 2.3 Upon completion of sanitization and set up (If applicable), place the R2S Sampling Head 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: Up to 50 feet of power supply cable and vacuum tubing can be employed if desired. Contact EM Technologies for additional information.
- 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 100-115 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 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/Reset button on the Remote Start Switch Assembly. Run the vacuum pump for a period of time, roughly 30-seconds to 1-minute, to purge the components residing within the domed area and sample conduit of the R2S of moisture and particulate matter from sanitization.
 - 2.6.2 Once the R2S unit has been sufficiently purged, press the Start/Stop/Reset button on the Remote-Start-Switch 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 60'00").

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- 2.7.1 Depressing the “up” or “down” arrow keypads 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/Reset assembly button before turning the unit power switch off.
 - 2.7.3 To reset to the preset time, hold down the Start/Stop/Reset button for approximately 3-seconds until the time resets.
- 2.8 Setting the flow rate to 60 SCFH:
- 2.8.1 Press the Start/Stop/Reset button to start the vacuum pump.
 - 2.8.2 Adjust the flow rate using the unit’s rotometer (airflow controller and gauge). To achieve an airflow of 60 Standard Cubic Feet per Hour (SCFH) 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 60 SCFH.
 - 2.8.3 Press the Start/Stop/Reset button 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 immediately prior to performing these steps.
- 2.9.1 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 R2S Sampling Assembly
 - Product Description, Lot Number and Step Number (if applicable)
 - 2.9.2 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 R2S.

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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.

- 2.9.3 Place the dome assembly down over the test plate and turntable.
- 2.9.4 Securely seat the dome flange in the “dome-to-base” seal and assure that the dome is properly seated in the seal. Press the dome down firmly and rotate slightly to assure a proper seal.
- 2.10 Adjust the Turntable / Test Plate Height Adjustment as follows:
 - 2.10.1 View the test plate distance indicator through the side of the dome to assure that test plate is at the proper height.
 - 2.10.2 Rotate the turntable adjustment knob clockwise or counter clockwise until the tip of the red distance indicator just touches the surface of the agar. This adjustment will assure the required distance of approximately 2-3 mm from the test plate surface to the opening of the sample slit.
- 2.11 Starting the Testing Period:
 - 2.11.1 Assure that the timer is set to the desired sample period then press the Start/Stop/Reset button 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 testing period can be terminated by pressing the Start/Stop/Reset button.
- 2.13 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.
 - 2.13.1 Remove the dome from the R2S 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.
 - 2.13.2 Remove the test plate, with the lid in place, from the turntable and place the dome back on the R2S.
 - 2.13.3 Secure the lid to the test plate with tape and document the “Sample Stop Time.”

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 and that they are reflective of the sampling period.

EXAMPLE:

The plate should make one full revolution if the timer is set for 60'00", 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.

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

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.).

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 dome assembly to remove any contaminants or test media residues on the sample throat or distance indicator.

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 R2S Sampling Assembly (i.e., on cart) 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.

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3. Storage and Transport**3.1 For transport convenience:**

- 3.1.1 The R2S should be placed in the transport mount located at the top of the R2S-C (controller) for Transport (and for storage).
- 3.1.2 The R2S vacuum and power supply assembly should be coiled and placed over the R2S 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 R2S Sampling Assembly in a clean and dry place.****3.4 A cover (i.e., bioshield or Wipe All) may be placed over the sample conduit of the R2S during transport and storage to minimize contamination.****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 as appropriate (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.

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- 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:

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

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 = 60 SCFH = 1 ft³ / minute

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

- 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 procedure.
- 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.