

CENTER OF EXCELLENCE FOR REGENERATIVE HEALTH BIOTECHNOLOGY EDUCATION CENTER		
SOP No. BEC-QC-001		Revision No. 00
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TITLE:

ENVIRONMENTAL MONITORING PROGRAM

CHANGE HISTORY:

REV. NO. – 00

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ENVIRONMENTAL MONITORING PROGRAM

1 PURPOSE

This document describes the environmental monitoring program for the Manufacturing Facility as well as the Quality Control methods and specifications for this program.

2 SCOPE

This procedure applies to all particulate and viable air monitoring, surface monitoring and personnel monitoring performed in the Manufacturing Facility. This program is in effect unless the Manufacturing Facility is in official shutdown.

3 RESPONSIBILITIES

It is the responsibility of Quality Control personnel to follow this procedure. It is the responsibility of supervisory personnel to ensure compliance with this procedure and to train employees responsible for performing this procedure.

4 DEFINITIONS

- 4.1 Biological Safety Cabinet (BSC) – a self-contained workstation with HEPA filtered air supply and exhaust.
- 4.2 Clean Room – a defined space equipped with air conditioning, air filtration, temperature and pressure controls as required, in which the concentration of airborne particles is controlled.
- 4.3 HEPA Air Filtration – high-efficiency particle air filtration where at least 99.7% of all particles 0.5 µm and larger are filtered from the air.
- 4.4 Alert Level – alert levels are quality levels that, when exceeded, signal a possible deviation from normal operating conditions and may not require action, but may need to be monitored more closely.
- 4.5 Action Level – action levels are quality levels that, when exceeded, signal an apparent deviation from normal operating conditions and requires immediate action.

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- 4.6 Class 100 – a defined area where the air quality meets the standard of a maximum of 100 particles 0.5 µm and larger per cubic foot of air; applies to biological safety cabinets (BSC). This environment is intended to provide direct isolation for sterile components from viable contamination while directly exposed to the environment. This includes the areas in which cells are cultured and viral particles are produced.
- 4.7 Class 10,000 – a defined area where the air quality meets the standard of a maximum of 10,000 particles 0.5 µm and larger per cubic foot of air. These environments are located immediately adjacent to and surrounding biosafety cabinets, where a requirement for low bioburden or low non-viable particle count is required by the process.
- 4.8 Class 100,000 – a defined area where the air quality meets the standard of a maximum of 100,000 particles 0.5 µm and larger per cubic foot of air.
- 4.9 At Rest –the description of the status of a clean room (facility), i.e., with all services functioning and with equipment functional, installed and operable or operating but without operating personnel in the facility.
- 4.10 Operational – the description of the status of a clean room (facility), in normal operation, with all services functioning and with equipment and personnel present and performing their normal work functions in the facility.
- 4.11 OOS – Out of specification

5 TEST AREAS

The Manufacturing Facility contains the following classified areas.

Biological Safety Cabinets			
Room Number	Air Quality	Suite Name	Description
403E			
BSC #1	100		
BSC #2	100		

Rooms				
Room No.	Room Name	Air Quality	Suite Name	Room Function
403	Classroom			Learning
403D	Gowning Room	100,000		Gowning
403E	Cleanroom	10,000		Production

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Classified areas are monitored on a routine basis during manufacturing campaigns. During shutdown, or inactive periods, monitoring may be reduced, or temporarily suspended by an appropriately approved deviation

6 ROUTINE MONITORING PROGRAM

6.1 The routine program is composed of the following types of monitoring:

- Non-Viable Airborne Particulates
- Viable Airborne Particulates
- Viable Surface Monitoring including the interior of the incubators, biosafety cabinets, and centrifuges
- Personnel Monitoring

6.2 Perform all scheduled environmental monitoring in one room before progressing to next room.

6.3 Perform particle counting, air sampling and RODAC plate sampling before placing settling plates.

7 REAGENTS AND MATERIALS

- Isopropyl alcohol, 70% (wash bottle)
- Rodac plates, TSA
- Settling plates, TSA
- Wipers, cleanroom
- Tape, laboratory
- EM room maps
- Appropriate forms
- Timer
- Marker
- Ladder
- Safety glasses
- Pen

8 STORAGE OF ENVIRONMENTAL MONITORING MEDIA

8.1 To place released environmental monitoring media in appropriate storage conditions, wipe outermost wrapping of plates or strips with 70% IPA or disinfectant. If wrapped with multiple layers, remove outermost wrapping.

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8.2 Transport into staging area. Store in released refrigerated storage area. Released media are stored within the facility according to SOP # QA-006.

9 DAILY PREPARATION FOR SAMPLING

- 9.1 Obtain appropriate Environmental Monitoring (EM) forms for work to be performed.
- 9.2 Wipe down cart, ladder, particle counter, and air sampler with wiper and 70% IPA or disinfectant. Do not allow sample port on counter to get wet. Place in degowning room on clean side of clean/dirty line.
- 9.3 Gown per SOP # FA-012 and enter the facility.
- 9.4 Obtain the appropriate number of released Rodac plates (SDA, TSA), and settling plates (TSA “14” cm) from refrigerated storage for the days monitoring.
 - 9.4.1 Include two plates of each type for negative controls.
 - 9.4.2 Include 2 plates for positive controls for SDA Rodac plates, 2 plates for positive controls for TSA Rodac plates, and 2 plates for positive controls for settling plates.
- 9.5 Examine plates for expiry dating, contamination or dry appearance. Do not open the plates to do this. Discard any that are out of date or which appear contaminated or dried out.
- 9.6 Record the manufacturer, receipt lot number, and expiry date for each type of media on the appropriate Environmental Monitoring Forms(s).
- 9.7 Allow plates to warm to room temperature (approximately 10 – 20 minutes).
- 9.8 Label each plate on the bottom with date, plate number, and sampler’s initials. Be certain to label prior to use.
- 9.9 Record test equipment identification information on the Monitoring forms as appropriate.
- 9.10 Transport the equipment, plates and strips on the cart from room to room as monitoring is performed.

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10 NON-VIABLE PARTICLE SAMPLING – OPERATION OF THE APC PARTICLE COUNTER

10.1 Particle sampling locations within each room are indicated in the attachments at the end of this SOP.

10.2 Select the appropriate operating options on the keypad including the particle size, the display mode (total particles, particles per cubic foot or particles per liter) and the sampling mode (single, automatic or continuous). Update the count time, date and time as necessary using the MODE key.

10.3 Use the following parameters

Particle size = 0.5 μ

Sampling mode = Single

Display Mode = /FT³

Count Time = one minute

10.4 Remove the isokinetic probe (the 3” long by ½” diameter black tube) from the carrying case and screw it into the top receptacle on the APC Plus. Be careful not to over tighten.

10.5 Power unit for at least 10 minutes before beginning to count.

10.6 Orient the sampling probe vertically during particle counting (except in horizontal laminar flow work stations).

10.7 Collect room samples approximately one foot above workstation height. Collect static hood samples approximately 6” above table top.

10.8 Collect three one minute samples at each location.

10.9 Record each one minute particle count, and the room temperature and humidity on APC Plus Particle Counter Data Sheet – Form # FM-048-QC-042

10.10 To begin counting, press the ON/OFF button to power up the unit. The LCD display should light up. The display will show the latest stored data prior to the last power off.

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- 10.11 Be careful not to depress the DOWN ARROW and the START/STOP keys simultaneously, this will clear all memory location in the APC Plus and resets the counter to Loc 1 for storage of the next data set.
- 10.12 After completion of the day's sampling, download the day's data from sampler to Excel spreadsheet. Print Excel spreadsheet, initial and date and attach to Particle Count Data Form. Save the file to C:\mydocuments\QCResults\QC042 on Computer EQ #066-2. Name the file QC042-YYYYMMDD-Initials.
- 10.13 If sampler printer is to be used:
- Connect the RS-232 cable to the printer and the particle counter. Make these connections with the APC Plus sampler and printer turned off.

11 RODAC PLATE SAMPLING PROCEDURE

- 11.1 Sampling locations are indicated on attachments.
- 11.2 Open one Rodac plate in a Class 10,000 room of the suite being monitored. Close the plate and label as "real time" negative controls. Use one plate of each media type, if both media are being used on that occasion.
- 11.3 Take surface samples with a Rodac plate, alternating 1 SDA or 1 TSA plate per location as indicated on room maps and according to the schedule.
- 11.3.1 At each location, remove the lid from the appropriately labeled plate and press gently onto the surface to be sampled. Do not rotate the plate while pressing.
- 11.4 When the sample has been taken, wipe the surface which has been sampled with a wiper and 70% IPA.
- 11.5 For sampling within the BSC, wipe the outer wrapper with alcohol or disinfectant then open in BSC. Place only the number of plates needed in the BSC. Wear sleeve protectors and sterile gloves when sampling in BSC.
- 11.6 Stack and tape together labeled plates to keep lids in place if needed. Place in the bag supplied or other container before removing them from the Manufacturing Facility.
- 11.7 In the QC Laboratory, inoculate the appropriate positive control plates for the media utilized. Inoculate one TSA Rodac plate with *B. subtilis*. Inoculate another TSA plate with *C. albicans*. Use Kwik-Stik control organisms. If only SDA Rodac plates

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were utilized during the days sampling, inoculate one positive control plate with *B. subtilis*. Inoculate another SDA plate with *C. albicans*. These plates will serve as positive controls for the day's sampling. In addition, include one unopened, unused plate as a negative control.

11.7.1 Tear open pouch and remove the Kwik-Stik.

11.7.2 Tear off Pull-Tab portion on label and attach to primary culture plate or QC record.

11.7.3 Pinch the bottom of the ampule in the cap with pliers to release the hydrating fluid.

11.7.4 Hold vertically and tap on counter to facilitate flow of fluid through shaft into bottom of unit containing pellet.

11.7.5 Crush the pellet and mix in fluid using a pinching action.

11.7.6 IMMEDIATELY saturate swab in hydrated suspension.

11.7.7 Inoculate the positive control plate(s) or strips by using pressure and rolling the swab in a circular area. Spread the organism over the surface of the plate.

11.8 Record the positive control lot information on appropriate environmental monitoring form.

12 SAMPLING PROCEDURE FOR SETTLING PLATES

12.1 Sampling locations are given in the room maps found in the Attachments.

12.2 Place the plate in the sampling location. Remove the lid and set aside. Vacate the area if monitoring is static.

12.2.1 Allow the plate to remain uncovered for at least 30 minutes, but no more than 4 hours, when monitoring class 10,000 and class 100, 000 rooms.

12.2.2 Allow the plate to sit uncovered for at least 1 hour, but no more than 4 hours, when monitoring class 100 areas (BSC).

12.3 At the end of the exposure period, cover plate.

12.4 Include one plate opened and closed vertically as a manipulated negative control.

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12.5 Once in the QC laboratory, inoculate one TSA plate with *B. subtilis* and one TSA plate with *C. albicans* to serve as a positive control for this day's sampling. See Kwik-Stik instructions in steps 11.7.1-11.7.7.

12.6 Include an unopened negative control plate for each sampling day.

13 INCUBATION OF PLATES

13.1 Incubate inverted TSA and SDA Rodac plates for 5-7 days at 20-25°C.

13.2 After the incubation period above, move the TSA Rodac plates for incubation for at least 2-3 days at 30-35°C. Record incubator equipment number, if appropriate, on all environmental monitoring forms.

13.3 Incubate inverted TSA settling plates for at least 2-3 days at 30-35°C .

13.4 Plate and strip readings that come due on weekends or holidays will be performed the next working day.

13.5 A preliminary colony count will be performed on day 2 or day 3, or the next working day, as appropriate during the incubation period for all types of media.

13.5.1 If preliminary counts are above the action level, discontinue incubation and proceed appropriately. If not, return all plates and strips to appropriate incubation temperature.

13.6 At the end of each incubation period, count the colonies found on each plate and record on appropriate form.

13.7 If the results reach the alert level or an OOS occurs, perform Gram stains on representative (phenotype) bacterial colonies recovered. Record Gram stain results on Form # FM-051-QC-043, Gram Stain Record. If unusual organisms are observed, discuss appropriate action with QC Management, Manufacturing Management and Quality Assurance.

13.8 If the results reach the alert level or an OOS occurs, identify molds to the genus level, if possible, by visual inspection of morphology using the unaided eye or under a dissecting microscope.

13.9 Notify QC Management, QA and Manufacturing Directors of all OOS occurrences by the next working day.

13.10 If molds are observed, notify QC Management, QA and Manufacturing Directors.

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14 SPECIFICATIONS

- 14.1 If a measurement meets or falls below the Action Level, the area is considered acceptable.
- 14.2 If during non-viable particulate monitoring, any measurement exceeds an Action Level, perform additional monitoring for up to a maximum of fifteen (15) one-minute readings.
 - 14.2.1 There must be five (5) consecutive 1 minute readings that meet or fall below the Action Level for the area to be considered acceptable.
- 14.3 An environmental excursion or out of specification (OOS) is defined as exceeding the Action Level in a single occurrence or exceeding the Alert Level on three consecutive samplings in the same area.

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Alert levels

Air Classification	Non Viable Particulate Count (0.5 µm particles/ft ³)	Rodac Plate Surface Counts (cfu/plate)	Settling Plates (cfu/14cm plate)
100	50 (at rest) 80 (operational)	2	1
10,000	8000	4	3
100,000	80,000	5	5

Action levels

Air Classification	Non Viable Particulate Count (0.5 µm particles/ft ³)	Rodac Plate Surface Counts (cfu/plate)	Settling Plates (cfu/14cm plate)
100	99 (at rest)	3	2
10,000	9999	5 10 for floors 20 floors dirty side	5
100,000	99999	20 30 for floors	20

Personnel Monitoring

Sample site	Alert	Action
Hand	1/plate	3/plate
Sleeve	1/plate	3/plate

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ATTACHMENT 1

