

Standard Operating Procedure      Allergenco D™ Analysis  
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## **Analytical Method for Qualitative Microbial Analysis of AllergencoD™ Bioaerosol Sampling Impactors**

### **1.0 Introduction**

The purpose of this protocol is to provide a standard procedure for the quantitative analysis of mold spores, pollen, skin fragments, insects, combustion particles, environmental dusts, and other aeroallergens for the AllergencoD™ sampling cassette. Although this protocol has been developed for use with the AllergencoD™ sampling cassette, use thereof, is not limited.

This protocol describes the method for conducting a quantitative analysis by qualified laboratory personnel. A basic review of the principles of operation, equipment, sample collection, removal and preparation as well as counting methodology, calculations, and counting rules are provided.

### **2.0 Principles of Operation**

The AllergencoD™ sampling cassette is patented under US patent No. 6,463,814,B1

The AllergencoD™ sampling cassette is an inertial impactor utilizing kinetic energy to impinge airborne particulate into an adhesive media. This type of impactor employs the use of a nozzle (or venturi) to accelerate air onto an adhesive coated glass substrate. The glass substrate is subsequently removed and the impaction zone (or trace) is then studied for quantitative analysis.

The AllergencoD™ contains a laminar flow venturi (patent pending) which directs airborne particles perpendicular to the plane of impaction. This creates a more well defined impaction trace resulting in more efficient collection and quantitative analysis.

### **3.0 Equipment**

Equipment required includes;

- AllergencoD™ sampling cassette
- Chain of Custody
- High volume vacuum supply
- Microscope slides (25mm x 75mm)
- Vinyl or Tygon tubing
- Glass cover slips
- Rotameter calibrated vs primary standard
- Lacto phenol staining reagent (if needed)
- Calibration master
- Stainless forceps or tweezers
- telescoping stand
- Microscope with 100x objective
- Permanent marker
- Low viscosity immersion oil
- Clean, dry sealable storage bag
- Stage micrometer
- Timer or stop watch

### **4.0 Sample Collection**

The AllergencoD™ sampling cassette is suitable for the collection of bioaerosols and aeroallergens employing industry standard sampling techniques. This type of instrument is suitable for the collection of airborne particulates such as mold spores, pollen, skin fragments, insects, combustion particles, environmental dusts, and other aeroallergens.

Vacuum source should be selected based on the capability to draw a minimum of 15 lpm through the cassette. Higher or lower flow rates are possible but may require adjustment to the sampling duration to prevent over or under loading respectively. Consult with Environmental Monitoring Systems, Inc. 800-293-3003 for suitable vacuum pumps from several manufacturers.

#### **4.1 Sample Calibration**

1. Use a rotameter with 1/4" O.D. tube fitting calibrated against a primary standard.
2. Directly connect a high volume vacuum source to a calibrated rotameter (if not previously provided) to the top side of the rotameter.
3. Use a suitable length of 1/4" ID vinyl or Tygon tubing.
4. Push end of the tubing on to the bottom fitting of the rotameter.
5. Remove protective label from the vacuum side (Bottom Quality Seal) of the cassette, retain for future use.
6. Attach opposite/free end of tubing to vacuum side (Bottom Quality Seal) of the cassette.
7. Remove the top protective label (AllergencoD™ Quality Seal) from the cassette, retain for future use.
8. Turn vacuum source on following manufacturer's procedure.
9. Adjust flow rate to achieve required flow rate (15 lpm typical) at the rotameter.
10. Lock the flow valve if appropriately fitted with locking nut.
11. Remove cassette and retain for future calibration procedure.

#### **4.2 Sampling Procedure**

Before starting any sampling procedure establish a sampling strategy for the environment being studied. Consult with a Certified Industrial Hygienist or qualified Environmental Engineer prior to the initiation of the investigation.

1. Remove the "Bottom Quality Seal" label from the cassette, retain for future use once sampling is completed.
2. Attach the calibrated vacuum supply via the vinyl/Tygon tubing to the cassette. The AllergencoD™ cassette is compatible with either 1/4" or 3/8" ID tubing.
3. Place the sampling cassette in the area of interest, typically on a telescopic stand.
4. Remove and retain the top protective "AllergencoD™ Quality Seal" label.
5. Using a stop watch or timer, turn your calibrated vacuum source and timer on simultaneously.
6. Conduct the sampling strategy according to the environment being studied (See below).
7. Once sampling duration has been achieved, turn vacuum source off.
8. Remove the cassette and replace protective labels.
9. Identify the sample accordingly using permanent marker.
10. Store appropriately until analysis can be conducted.

#### **4.3 Sampling Strategy**

Sampling strategy should account for the environment being studied, clean dust free indoor environments can run for longer durations. Higher flow rates tend to reduce the collection efficiency of smaller particles (i.e. less than 3 microns) while lower flow rates tend to reduce collection of larger particles. A guide for sampling duration is provided below.

| <b><i>Recommended Sampling Durations for the<br/>AllergencoD™<br/>Air Sampling Cassette</i></b> |                  |                 |
|---|------------------|-----------------|
| <b>Environmental Condition</b>  | <b>Flow Rate</b> | <b>Duration</b> |
| Outdoor sample  | 15 lpm           | 8-10 minutes    |
| Dust free environment (clean office)  | 15 lpm           | 5-8 minutes     |
| Indoor environment - occupied space   | 15 lpm           | 3-5 minutes     |
| Indoor environment - excessive dust   | 15 lpm           | 1-3 minutes     |
| Inner Wall Sampling   | 15 lpm           | 1-3 minutes     |

A chain of custody (COC) should be used to document the sample rate and duration as well as to identify the specific location of the samples collected. A prepared COC shall be provided to the laboratory conducting the analysis upon submission of the samples.

Samples should be submitted to a qualified laboratory.

## **5.0 Sample Removal and Preparation**

Cassettes should be received with the outer labels and Quality Seals intact, tampering with this label shall void the sample. All samples shall be accompanied by an appropriate Chain of Custody

Bioaerosol sampling cassettes contain a small, typically glass slide, substrate, that supports the adhesive media. This coated glass slide must be removed to conduct analytical studies. The center portion of this coated glass slide will contain an impaction trace which is typically visible to the naked eye. This impaction trace is approximately 1.1 mm wide by 14.4mm long.

1. Obtain and set aside a pre-cleaned 25mm x 75mm standard microscope slide.
2. Remove or cut the circumferential protective band that seals and retains that upper and lower portions together.
3. With the cassette placed on a stable work surface, insert a coin, flat screwdriver or suitable tool, into the slot separating the two halves of the cassette and gently pry apart at several locations around the circumference. Carefully remove the top half, allowing the coated glass slide to remain in the lower portion of the cassette.
4. Carefully remove the coated glass slide using tweezers or suitable tool.
5. Place on to the standard 25mm x 75mm microscope slide with the exposed adhesive side up.
6. Orient the coated glass slide so the impaction trace runs parallel with the length of the microscope slide.
7. Place a bead of contact adhesive (or clear fingernail polish) around the perimeter of the coated slide this will prevent the sample from moving during analysis.
8. Place a small drop of Lacto phenol Cotton Blue stain just slightly off the corner of the trace on the

coated slide—do not apply staining solution directly on the impaction trace as it may “wash away” particles making analysis difficult.

9. Carefully place the edge of the cover slip adjacent to the impaction trace and lay the cover slip on top of the coated slide. This will spread the staining agent over the trace area.
10. Place a small drop of low viscosity immersion oil on top of the cover slip near the center.
11. Carefully place the 25mm x 75mm standard microscope slide into the mechanical stage of the microscope.
12. Read the prepared sample using a 100x oil immersion objective.

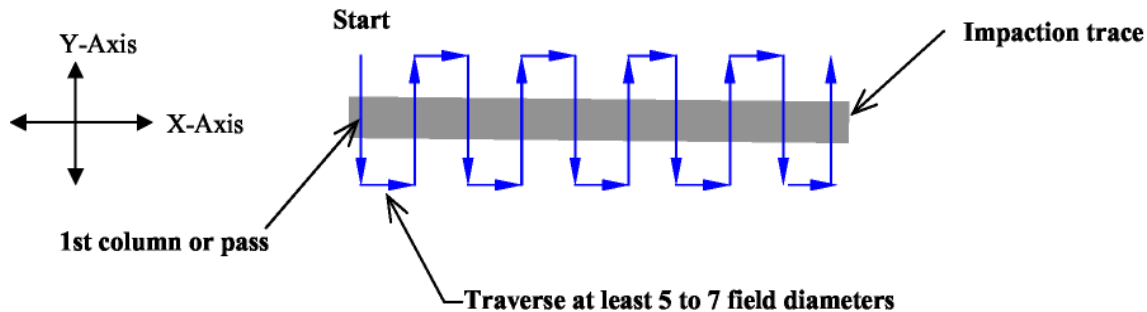
Note: The use of a staining agent such as Lacto Phenol Cotton Blue is not required and is left to the discretion of the laboratory conducting the analysis.

## 6.0 Counting Methodology

The most accurate counting method requires the observation of 100% of the impaction trace, however, this practice may not be practical and is dependent on the laboratory conducting the analysis. The counting method described herein uses a microscope with a 100x oil immersion objective.

The impaction trace is approximately 1.1 mm wide by 14.4 mm long. Observations will be made in columns across the entire width (Y-axis) of the impaction trace followed by subsequent observations traversing the length (X-axis) of the trace. A total of 10 passes or traverses (minimum) will be taken across the X-axis of the trace.

1. Scan the sample to find the impaction trace.
2. Adjust the mechanical stage such that the start is near the left side of the impaction trace.
3. Adjust the stage so the field diameter reveals the entire upper left corner of the impaction trace.
4. Starting in the upper right quadrant of the field of view, observe the sample in a clockwise rotation.
5. Using a tally sheet, identify, count and record the identity of each particle observed.
6. While analyzing each quadrant, adjust the focal depth to view particles that may exist in different planes.
7. Adjust the Y-axis stage to observe the next field of view, do not move in the X-axis until the pass is entirely completed.
8. Again, starting in the upper right quadrant of the field of view, observe the sample in a clockwise rotation.
9. Using a tally sheet, identify, count and record the identity of each particle observed.
10. Repeat steps #4 through #7 until the entire column has been observed and recorded.
11. Move the X-axis stage to the left (view moves right) several field diameters—this may vary depending on the field diameter, the procedure should spread observations about the entire X-axis length.
12. Repeat Steps #4 through #11 until the desired degree of confidence is achieved. i.e. Counting 1 of 5 traverses will read 20% of the trace. Some laboratories find a minimum total of 10 passes or traverses to be sufficient, other laboratories observe and record 100% of the trace.



## 7.0 Calculations

The purpose of the analysis is to provide data in the form of **Spore per cubic meter (S/m<sup>3</sup>)**. To conduct the calculation the following information is required:

Impactation trace width = 1.1 mm

Impactation trace length = 14.4 mm

Air flow rate = 15 liters per minute (typical)

= .015 m<sup>3</sup>/min

Number of traverses = 10 minimum

Sampling duration = in minutes (typically 1 to 10 minutes depending on environment)

Field diameter = in mm (for 100x objective) using a stage micrometer

The formula used to obtain Spores/m<sup>3</sup> is as follows:

Spores/m<sup>3</sup> = Number of spore counted / Volume analyzed

Volume analyzed = Fraction counted x Volume tested

Fraction counted = (Field diameter x Number of traverses) / Trace length

Volume tested = Flow rate x Duration

For an example, using the following assumptions:

Field diameter = .180mm (based on stage micrometer reading)

Trace length = 14.4mm

Number of traverses = 10

Flow rate = .015m<sup>3</sup>/min

Duration = 8 min

Fraction counted = (.180mm x 10) / 14.4mm = 1.80mm / 14.4mm = .125

Volume tested = .015m<sup>3</sup>/min x 8 minutes = 0.12 m<sup>3</sup>

Volume analyzed = Fraction counted x Volume tested = .125 x 0.12 m<sup>3</sup> = .015 m<sup>3</sup>

If one (1) spore is counted then:

$$\text{Spores/m}^3 = 1 / .015 = 66.6 \text{ Spores / m}^3$$

If two (2) spores are counted then:

$$\text{Spore/m}^3 = 2 / .015 = 133.33 \text{ Spore / m}^3$$

If twelve (12) spores are counted then:

$$\text{Spores/m}^3 = 12 / .015 = 800 \text{ Spores / m}^3$$

## 7.0 Calculations cont

As previously reviewed, the Volume analyzed is dependent on:

- Flow rate of 15 lpm
- Sampling duration (variable)
- Field diameter (variable)
- Number of traverses/passes (variable)
- Trace length of 14.4 mm

The following charts include values for “Volume analyzed” using the standard flow rate of 15lpm. Each chart represents a specific sampling duration (from 1 to 10 minutes) with varying values for Field diameter and number of traverses/passes (from 10 to 30 traverses)

Using the basic formula for calculating Spores per Cubic Meter:  $\text{Spores/m}^3 = \text{Number of spores counted} / \text{Volume analyzed}$

1. Select the appropriate chart for the sampling duration used (from 10 to 1 minute).
2. Locate the Field diameter in the left column corresponding to your stage micrometer reading.
3. Move across the row to the column corresponding to the number of traverses used during counting.
4. Record the value for “Volume analyzed” where these values intersect.
5. Apply the formula:  $\text{Spores/m}^3 = \text{Number of spores counted} / \text{Volume analyzed}$ .

**"Volume analyzed" for Sampling duration = 10 minutes Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0167 | 0.0200 | 0.0233 | 0.0267 | 0.0300 | 0.0333 | 0.0367 | 0.0400 | 0.0433 | 0.0500 |
| 0.165          | 0.0172 | 0.0206 | 0.0241 | 0.0275 | 0.0309 | 0.0344 | 0.0378 | 0.0413 | 0.0447 | 0.0516 |
| 0.170          | 0.0177 | 0.0213 | 0.0248 | 0.0283 | 0.0319 | 0.0354 | 0.0390 | 0.0425 | 0.0460 | 0.0531 |
| 0.175          | 0.0182 | 0.0219 | 0.0255 | 0.0292 | 0.0328 | 0.0365 | 0.0401 | 0.0380 | 0.0474 | 0.0547 |
| 0.180          | 0.0188 | 0.0225 | 0.0263 | 0.0300 | 0.0338 | 0.0375 | 0.0413 | 0.0450 | 0.0488 | 0.0563 |
| 0.185          | 0.0193 | 0.0231 | 0.0270 | 0.0308 | 0.0347 | 0.0385 | 0.0424 | 0.0463 | 0.0501 | 0.0578 |
| 0.190          | 0.0198 | 0.0238 | 0.0277 | 0.0317 | 0.0356 | 0.0396 | 0.0435 | 0.0475 | 0.0515 | 0.0594 |
| 0.195          | 0.0203 | 0.0244 | 0.0284 | 0.0325 | 0.0366 | 0.0406 | 0.0447 | 0.0488 | 0.0528 | 0.0609 |
| 0.200          | 0.0208 | 0.0250 | 0.0292 | 0.0333 | 0.0375 | 0.0417 | 0.0458 | 0.0500 | 0.0542 | 0.0625 |
| 0.205          | 0.0214 | 0.0256 | 0.0299 | 0.0342 | 0.0384 | 0.0427 | 0.0470 | 0.0513 | 0.0555 | 0.0641 |
| 0.210          | 0.0219 | 0.0263 | 0.0306 | 0.0350 | 0.0394 | 0.0438 | 0.0481 | 0.0525 | 0.0569 | 0.0656 |
| 0.215          | 0.0224 | 0.0269 | 0.0314 | 0.0358 | 0.0403 | 0.0448 | 0.0493 | 0.0538 | 0.0582 | 0.0672 |
| 0.220          | 0.0229 | 0.0275 | 0.0321 | 0.0367 | 0.0413 | 0.0458 | 0.0504 | 0.0550 | 0.0596 | 0.0688 |
| 0.225          | 0.0234 | 0.0281 | 0.0328 | 0.0375 | 0.0422 | 0.0469 | 0.0516 | 0.0563 | 0.0609 | 0.0703 |

**"Volume analyzed" for Sampling duration = 9 minutes  
 Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0150 | 0.0180 | 0.0210 | 0.0240 | 0.0270 | 0.0300 | 0.0330 | 0.0360 | 0.0390 | 0.0450 |
| 0.165          | 0.0155 | 0.0186 | 0.0217 | 0.0248 | 0.0278 | 0.0309 | 0.0340 | 0.0371 | 0.0402 | 0.0464 |
| 0.170          | 0.0159 | 0.0191 | 0.0223 | 0.0255 | 0.0287 | 0.0319 | 0.0351 | 0.0383 | 0.0414 | 0.0478 |
| 0.175          | 0.0164 | 0.0197 | 0.0230 | 0.0263 | 0.0295 | 0.0328 | 0.0361 | 0.0394 | 0.0427 | 0.0492 |
| 0.180          | 0.0169 | 0.0203 | 0.0236 | 0.0270 | 0.0304 | 0.0338 | 0.0371 | 0.0405 | 0.0439 | 0.0506 |
| 0.185          | 0.0173 | 0.0280 | 0.0243 | 0.0278 | 0.0312 | 0.0347 | 0.0382 | 0.0416 | 0.0451 | 0.0520 |
| 0.190          | 0.0178 | 0.0410 | 0.0249 | 0.0285 | 0.0321 | 0.0356 | 0.0392 | 0.0428 | 0.0463 | 0.0534 |
| 0.195          | 0.0183 | 0.0219 | 0.0256 | 0.0293 | 0.0329 | 0.0366 | 0.0402 | 0.0439 | 0.0475 | 0.0548 |
| 0.200          | 0.0188 | 0.0225 | 0.0263 | 0.0300 | 0.0338 | 0.0375 | 0.0413 | 0.0450 | 0.0488 | 0.0563 |
| 0.205          | 0.0192 | 0.0231 | 0.0269 | 0.0308 | 0.0346 | 0.0384 | 0.0423 | 0.0461 | 0.0500 | 0.0577 |
| 0.210          | 0.0197 | 0.0236 | 0.0276 | 0.0315 | 0.0354 | 0.0394 | 0.0433 | 0.0473 | 0.0512 | 0.0591 |
| 0.215          | 0.0202 | 0.0242 | 0.0282 | 0.0323 | 0.0363 | 0.0403 | 0.0443 | 0.0484 | 0.0524 | 0.0605 |
| 0.220          | 0.0206 | 0.0248 | 0.0289 | 0.0330 | 0.0371 | 0.0413 | 0.0454 | 0.0495 | 0.0536 | 0.0619 |
| 0.225          | 0.0211 | 0.0253 | 0.0295 | 0.0338 | 0.0380 | 0.0422 | 0.0464 | 0.0506 | 0.0548 | 0.0633 |

**"Volume Analyzed" for Sample duration = 8 minutes**  
 Number of traverses (passes)

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0133 | 0.0160 | 0.0187 | 0.0213 | 0.0240 | 0.0267 | 0.0293 | 0.0320 | 0.0347 | 0.0400 |
| 0.165          | 0.0138 | 0.0165 | 0.0193 | 0.0220 | 0.0248 | 0.0275 | 0.0303 | 0.0330 | 0.0358 | 0.0413 |
| 0.170          | 0.0142 | 0.0170 | 0.0198 | 0.0227 | 0.0255 | 0.0283 | 0.0312 | 0.0340 | 0.0368 | 0.0425 |
| 0.175          | 0.0146 | 0.0175 | 0.0204 | 0.0233 | 0.0263 | 0.0292 | 0.0321 | 0.0350 | 0.0379 | 0.0438 |
| 0.180          | 0.0150 | 0.0180 | 0.0210 | 0.0240 | 0.0270 | 0.0300 | 0.0330 | 0.0360 | 0.0390 | 0.0450 |
| 0.185          | 0.0154 | 0.0185 | 0.0216 | 0.0247 | 0.0278 | 0.0308 | 0.0339 | 0.0370 | 0.0401 | 0.0463 |
| 0.190          | 0.0158 | 0.0190 | 0.0222 | 0.0253 | 0.0285 | 0.0317 | 0.0348 | 0.0380 | 0.0412 | 0.0475 |
| 0.195          | 0.0163 | 0.0195 | 0.0228 | 0.0260 | 0.0293 | 0.0325 | 0.0358 | 0.0390 | 0.0423 | 0.0488 |
| 0.200          | 0.0167 | 0.0200 | 0.0233 | 0.0267 | 0.0300 | 0.0333 | 0.0367 | 0.0400 | 0.0433 | 0.0500 |
| 0.205          | 0.0171 | 0.0205 | 0.0239 | 0.0273 | 0.0308 | 0.0342 | 0.0376 | 0.0410 | 0.0444 | 0.0513 |
| 0.210          | 0.0175 | 0.0210 | 0.0245 | 0.0280 | 0.0315 | 0.0350 | 0.0385 | 0.0420 | 0.0455 | 0.0525 |
| 0.215          | 0.0179 | 0.0215 | 0.0251 | 0.0287 | 0.0323 | 0.0358 | 0.0394 | 0.0430 | 0.0466 | 0.0538 |
| 0.220          | 0.0183 | 0.0220 | 0.0257 | 0.0293 | 0.0330 | 0.0367 | 0.0403 | 0.0440 | 0.0477 | 0.0550 |
| 0.225          | 0.0188 | 0.0225 | 0.0263 | 0.0300 | 0.0338 | 0.0375 | 0.0413 | 0.0450 | 0.0488 | 0.0563 |



**"Volume Analyzed" for Sample duration =7 minutes**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0117 | 0.0140 | 0.0163 | 0.0187 | 0.0210 | 0.0233 | 0.0257 | 0.0280 | 0.0303 | 0.0350 |
| 0.165          | 0.0120 | 0.0144 | 0.0168 | 0.0193 | 0.0217 | 0.0241 | 0.0265 | 0.0289 | 0.0313 | 0.0361 |
| 0.170          | 0.0124 | 0.0149 | 0.0174 | 0.0198 | 0.0223 | 0.0248 | 0.0273 | 0.0298 | 0.0322 | 0.0372 |
| 0.175          | 0.0128 | 0.0153 | 0.0179 | 0.0204 | 0.0230 | 0.0255 | 0.0281 | 0.0306 | 0.0332 | 0.0383 |
| 0.180          | 0.0131 | 0.0158 | 0.0184 | 0.0210 | 0.0236 | 0.0263 | 0.0289 | 0.0315 | 0.0341 | 0.0394 |
| 0.185          | 0.0135 | 0.0162 | 0.0189 | 0.0216 | 0.0243 | 0.0270 | 0.0297 | 0.0324 | 0.0351 | 0.0405 |
| 0.190          | 0.0139 | 0.0166 | 0.0194 | 0.0222 | 0.0249 | 0.0277 | 0.0305 | 0.0333 | 0.0360 | 0.0416 |
| 0.195          | 0.0142 | 0.0171 | 0.0199 | 0.0228 | 0.0256 | 0.0284 | 0.0313 | 0.0341 | 0.0370 | 0.0427 |
| 0.200          | 0.0146 | 0.0175 | 0.0204 | 0.0233 | 0.0263 | 0.0292 | 0.0321 | 0.0350 | 0.0379 | 0.0438 |
| 0.205          | 0.0149 | 0.0179 | 0.0209 | 0.0239 | 0.0269 | 0.0299 | 0.0329 | 0.0359 | 0.0389 | 0.0448 |
| 0.210          | 0.0153 | 0.0184 | 0.0214 | 0.0245 | 0.0276 | 0.0306 | 0.0337 | 0.0368 | 0.0398 | 0.0459 |
| 0.215          | 0.0157 | 0.0188 | 0.0219 | 0.0251 | 0.0282 | 0.0314 | 0.0345 | 0.0376 | 0.0408 | 0.0470 |
| 0.220          | 0.0160 | 0.0193 | 0.0225 | 0.0257 | 0.0289 | 0.0321 | 0.0353 | 0.0385 | 0.0417 | 0.0481 |
| 0.225          | 0.0164 | 0.0197 | 0.0230 | 0.0263 | 0.0295 | 0.0328 | 0.0361 | 0.0394 | 0.0427 | 0.0492 |

**"Volume Analyzed" for Sample duration = 6 minutes**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0100 | 0.0120 | 0.0140 | 0.0160 | 0.0180 | 0.0200 | 0.0220 | 0.0240 | 0.0260 | 0.0300 |
| 0.165          | 0.0103 | 0.0124 | 0.0144 | 0.0165 | 0.0186 | 0.2060 | 0.0227 | 0.0248 | 0.0268 | 0.0309 |
| 0.170          | 0.0106 | 0.0128 | 0.0149 | 0.0170 | 0.0191 | 0.0213 | 0.0234 | 0.0255 | 0.0276 | 0.0319 |
| 0.175          | 0.0109 | 0.0131 | 0.0153 | 0.0175 | 0.0197 | 0.0219 | 0.0241 | 0.0263 | 0.0284 | 0.0328 |
| 0.180          | 0.0113 | 0.0135 | 0.0158 | 0.0180 | 0.0203 | 0.0225 | 0.0248 | 0.0270 | 0.0293 | 0.0338 |
| 0.185          | 0.0116 | 0.0139 | 0.0162 | 0.0185 | 0.0208 | 0.0231 | 0.0254 | 0.0278 | 0.0301 | 0.0347 |
| 0.190          | 0.0119 | 0.0143 | 0.0166 | 0.0190 | 0.0214 | 0.0238 | 0.0261 | 0.0285 | 0.0309 | 0.0356 |
| 0.195          | 0.0122 | 0.0146 | 0.0171 | 0.0195 | 0.0219 | 0.0244 | 0.0268 | 0.0293 | 0.0317 | 0.0366 |
| 0.200          | 0.0125 | 0.0150 | 0.0175 | 0.0200 | 0.0225 | 0.0250 | 0.0275 | 0.0300 | 0.0325 | 0.0375 |
| 0.205          | 0.0128 | 0.0154 | 0.0179 | 0.0205 | 0.0231 | 0.0256 | 0.0282 | 0.0308 | 0.0333 | 0.0384 |
| 0.210          | 0.0131 | 0.0158 | 0.0184 | 0.0210 | 0.0236 | 0.0263 | 0.0289 | 0.0315 | 0.0341 | 0.0394 |
| 0.215          | 0.0134 | 0.0161 | 0.0188 | 0.0215 | 0.0242 | 0.0269 | 0.0296 | 0.0323 | 0.0349 | 0.0403 |
| 0.220          | 0.0138 | 0.0165 | 0.0193 | 0.0220 | 0.0248 | 0.0275 | 0.0303 | 0.0330 | 0.0358 | 0.0413 |
| 0.225          | 0.0141 | 0.0169 | 0.0197 | 0.0225 | 0.0253 | 0.0281 | 0.0309 | 0.0338 | 0.0366 | 0.0422 |

**"Volume Analyzed" for Sample duration = 5 minutes**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0083 | 0.0100 | 0.0117 | 0.0133 | 0.0150 | 0.0167 | 0.0183 | 0.0200 | 0.0217 | 0.0250 |
| 0.165          | 0.0086 | 0.0103 | 0.0120 | 0.0138 | 0.0155 | 0.0172 | 0.0189 | 0.0206 | 0.0223 | 0.0258 |
| 0.170          | 0.0089 | 0.0106 | 0.0124 | 0.0142 | 0.0159 | 0.0177 | 0.0195 | 0.0213 | 0.0230 | 0.0266 |
| 0.175          | 0.0091 | 0.0109 | 0.0128 | 0.0146 | 0.0164 | 0.0182 | 0.0201 | 0.0219 | 0.0237 | 0.0273 |
| 0.180          | 0.0094 | 0.0113 | 0.0131 | 0.0150 | 0.0169 | 0.0188 | 0.0206 | 0.0225 | 0.0244 | 0.0281 |
| 0.185          | 0.0096 | 0.0116 | 0.0135 | 0.0154 | 0.0173 | 0.0193 | 0.0212 | 0.0231 | 0.0251 | 0.0289 |
| 0.190          | 0.0099 | 0.0119 | 0.0139 | 0.0158 | 0.0178 | 0.0198 | 0.0218 | 0.0238 | 0.0257 | 0.0297 |
| 0.195          | 0.0102 | 0.0122 | 0.0142 | 0.0163 | 0.0183 | 0.0203 | 0.0223 | 0.0244 | 0.0264 | 0.0305 |
| 0.200          | 0.0104 | 0.0125 | 0.0146 | 0.0167 | 0.0188 | 0.0208 | 0.0229 | 0.0250 | 0.0271 | 0.0313 |
| 0.205          | 0.0107 | 0.0128 | 0.0149 | 0.0171 | 0.0192 | 0.0214 | 0.0235 | 0.0256 | 0.0278 | 0.0320 |
| 0.210          | 0.0109 | 0.0131 | 0.0153 | 0.0175 | 0.0197 | 0.0219 | 0.0241 | 0.0263 | 0.0284 | 0.0328 |
| 0.215          | 0.0112 | 0.0134 | 0.0157 | 0.0179 | 0.0202 | 0.0224 | 0.0246 | 0.0269 | 0.0291 | 0.0336 |
| 0.220          | 0.0115 | 0.0138 | 0.0160 | 0.0183 | 0.0206 | 0.0229 | 0.0252 | 0.0275 | 0.0298 | 0.0344 |
| 0.225          | 0.0117 | 0.0141 | 0.0164 | 0.0188 | 0.0211 | 0.0234 | 0.0258 | 0.0281 | 0.0305 | 0.0352 |

**"Volume Analyzed" for Sample duration = 4 minutes**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0067 | 0.008  | 0.0093 | 0.0107 | 0.012  | 0.0133 | 0.0147 | 0.016  | 0.0173 | 0.02   |
| 0.165          | 0.0069 | 0.0083 | 0.0096 | 0.011  | 0.0124 | 0.0138 | 0.0151 | 0.0165 | 0.0179 | 0.0206 |
| 0.170          | 0.0071 | 0.0085 | 0.0099 | 0.0113 | 0.0128 | 0.0142 | 0.0156 | 0.017  | 0.0184 | 0.0213 |
| 0.175          | 0.0073 | 0.0088 | 0.0102 | 0.0117 | 0.0131 | 0.0146 | 0.016  | 0.0175 | 0.019  | 0.0219 |
| 0.180          | 0.0075 | 0.009  | 0.0105 | 0.012  | 0.0135 | 0.015  | 0.0165 | 0.018  | 0.0195 | 0.0225 |
| 0.185          | 0.0077 | 0.0093 | 0.0108 | 0.0123 | 0.0139 | 0.0154 | 0.017  | 0.0185 | 0.02   | 0.0231 |
| 0.190          | 0.0079 | 0.0095 | 0.0111 | 0.0127 | 0.0143 | 0.0158 | 0.0174 | 0.019  | 0.0206 | 0.0238 |
| 0.195          | 0.0081 | 0.0098 | 0.0114 | 0.013  | 0.0146 | 0.0163 | 0.0179 | 0.0195 | 0.0211 | 0.0244 |
| 0.200          | 0.0083 | 0.01   | 0.0117 | 0.0133 | 0.015  | 0.0167 | 0.0183 | 0.02   | 0.0217 | 0.025  |
| 0.205          | 0.0085 | 0.0103 | 0.012  | 0.0137 | 0.0154 | 0.0171 | 0.0188 | 0.0205 | 0.0222 | 0.0256 |
| 0.210          | 0.0088 | 0.0105 | 0.0123 | 0.014  | 0.0158 | 0.0175 | 0.0193 | 0.021  | 0.0228 | 0.0263 |
| 0.215          | 0.009  | 0.0108 | 0.0125 | 0.0143 | 0.0161 | 0.0179 | 0.0197 | 0.0215 | 0.0233 | 0.0269 |
| 0.220          | 0.0092 | 0.011  | 0.0128 | 0.0147 | 0.0165 | 0.0183 | 0.0202 | 0.022  | 0.0238 | 0.0275 |
| 0.225          | 0.0094 | 0.0113 | 0.0131 | 0.015  | 0.0169 | 0.0188 | 0.0206 | 0.0225 | 0.0244 | 0.0281 |

**"Volume Analyzed" for Sample duration = 3 minutes**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0050 | 0.0060 | 0.0070 | 0.0080 | 0.0090 | 0.0100 | 0.0110 | 0.0120 | 0.0130 | 0.0150 |
| 0.165          | 0.0052 | 0.0062 | 0.0072 | 0.0083 | 0.0093 | 0.0103 | 0.0113 | 0.0124 | 0.0134 | 0.0155 |
| 0.170          | 0.0053 | 0.0064 | 0.0074 | 0.0085 | 0.0096 | 0.0106 | 0.0117 | 0.0128 | 0.0138 | 0.0159 |
| 0.175          | 0.0055 | 0.0066 | 0.0077 | 0.0088 | 0.0098 | 0.0109 | 0.0120 | 0.0131 | 0.0142 | 0.0164 |
| 0.180          | 0.0056 | 0.0068 | 0.0079 | 0.0090 | 0.0101 | 0.0113 | 0.0124 | 0.0135 | 0.0146 | 0.0169 |
| 0.185          | 0.0058 | 0.0069 | 0.0081 | 0.0093 | 0.0104 | 0.0116 | 0.0127 | 0.0139 | 0.0150 | 0.0173 |
| 0.190          | 0.0059 | 0.0071 | 0.0083 | 0.0095 | 0.0107 | 0.0119 | 0.0131 | 0.0143 | 0.0154 | 0.0178 |
| 0.195          | 0.0061 | 0.0073 | 0.0085 | 0.0098 | 0.0110 | 0.0122 | 0.0134 | 0.0146 | 0.0158 | 0.0183 |
| 0.200          | 0.0063 | 0.0075 | 0.0088 | 0.0100 | 0.0113 | 0.0125 | 0.0138 | 0.0150 | 0.0163 | 0.0188 |
| 0.205          | 0.0064 | 0.0077 | 0.0090 | 0.0103 | 0.0115 | 0.0128 | 0.0141 | 0.0154 | 0.0167 | 0.0192 |
| 0.210          | 0.0066 | 0.0079 | 0.0092 | 0.0105 | 0.0118 | 0.0131 | 0.0144 | 0.0158 | 0.0171 | 0.0197 |
| 0.215          | 0.0067 | 0.0081 | 0.0094 | 0.0108 | 0.0121 | 0.0134 | 0.0148 | 0.0161 | 0.0175 | 0.0202 |
| 0.220          | 0.0069 | 0.0083 | 0.0096 | 0.0110 | 0.0124 | 0.0138 | 0.0151 | 0.0165 | 0.0179 | 0.0206 |
| 0.225          | 0.0070 | 0.0084 | 0.0098 | 0.0113 | 0.0127 | 0.0141 | 0.0155 | 0.0169 | 0.0183 | 0.0211 |

**"Volume Analyzed" for Sample duration = 2 minutes**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0033 | 0.004  | 0.0047 | 0.0053 | 0.006  | 0.0067 | 0.0073 | 0.008  | 0.0087 | 0.01   |
| 0.165          | 0.0034 | 0.0041 | 0.0048 | 0.0055 | 0.0062 | 0.0069 | 0.0076 | 0.0083 | 0.0089 | 0.0103 |
| 0.170          | 0.0035 | 0.0043 | 0.005  | 0.0057 | 0.0064 | 0.0071 | 0.0078 | 0.0085 | 0.0092 | 0.0106 |
| 0.175          | 0.0036 | 0.0044 | 0.0051 | 0.0058 | 0.0066 | 0.0073 | 0.008  | 0.0088 | 0.0095 | 0.0109 |
| 0.180          | 0.0038 | 0.0045 | 0.0053 | 0.006  | 0.0068 | 0.0075 | 0.0083 | 0.009  | 0.0098 | 0.0113 |
| 0.185          | 0.0039 | 0.0046 | 0.0054 | 0.0062 | 0.0069 | 0.0077 | 0.0085 | 0.0093 | 0.01   | 0.0116 |
| 0.190          | 0.004  | 0.0048 | 0.0055 | 0.0063 | 0.0071 | 0.0079 | 0.0087 | 0.0095 | 0.0103 | 0.0119 |
| 0.195          | 0.0041 | 0.0049 | 0.0057 | 0.0065 | 0.0073 | 0.0081 | 0.0089 | 0.0098 | 0.0106 | 0.0122 |
| 0.200          | 0.0042 | 0.005  | 0.0058 | 0.0067 | 0.0075 | 0.0083 | 0.0092 | 0.01   | 0.0108 | 0.0125 |
| 0.205          | 0.0043 | 0.0051 | 0.006  | 0.0068 | 0.0077 | 0.0085 | 0.0094 | 0.0103 | 0.0111 | 0.0128 |
| 0.210          | 0.0044 | 0.0053 | 0.0061 | 0.007  | 0.0079 | 0.0088 | 0.0096 | 0.0105 | 0.0114 | 0.0131 |
| 0.215          | 0.0045 | 0.0054 | 0.0063 | 0.0072 | 0.0081 | 0.009  | 0.0099 | 0.0108 | 0.0116 | 0.0134 |
| 0.220          | 0.0046 | 0.0055 | 0.0064 | 0.0073 | 0.0083 | 0.0092 | 0.0101 | 0.011  | 0.0119 | 0.0138 |
| 0.225          | 0.0047 | 0.0056 | 0.0066 | 0.0075 | 0.0084 | 0.0094 | 0.0103 | 0.0113 | 0.0122 | 0.0141 |

**"Volume Analyzed" for Sample duration = 1 minute**  
**Number of traverses (passes)**

| Field Diameter | 10     | 12     | 14     | 16     | 18     | 20     | 22     | 24     | 26     | 30     |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.160          | 0.0017 | 0.002  | 0.0023 | 0.0027 | 0.003  | 0.0033 | 0.0037 | 0.004  | 0.0043 | 0.005  |
| 0.165          | 0.0017 | 0.0021 | 0.0024 | 0.0028 | 0.0031 | 0.0034 | 0.0038 | 0.0041 | 0.0045 | 0.0052 |
| 0.170          | 0.0018 | 0.0021 | 0.0025 | 0.0028 | 0.0032 | 0.0035 | 0.0039 | 0.0043 | 0.0046 | 0.0053 |
| 0.175          | 0.0018 | 0.0022 | 0.0026 | 0.0029 | 0.0033 | 0.0036 | 0.004  | 0.0044 | 0.0047 | 0.0055 |
| 0.180          | 0.0019 | 0.0023 | 0.0026 | 0.003  | 0.0034 | 0.0038 | 0.0041 | 0.0045 | 0.0049 | 0.0056 |
| 0.185          | 0.0019 | 0.0023 | 0.0027 | 0.0031 | 0.0035 | 0.0039 | 0.0042 | 0.0046 | 0.005  | 0.0058 |
| 0.190          | 0.002  | 0.0024 | 0.0028 | 0.0032 | 0.0036 | 0.004  | 0.0044 | 0.0048 | 0.0051 | 0.0059 |
| 0.195          | 0.002  | 0.0024 | 0.0028 | 0.0033 | 0.0037 | 0.0041 | 0.0045 | 0.0049 | 0.0053 | 0.0061 |
| 0.200          | 0.0021 | 0.0025 | 0.0029 | 0.0033 | 0.0038 | 0.0042 | 0.0046 | 0.005  | 0.0054 | 0.0063 |
| 0.205          | 0.0021 | 0.0026 | 0.003  | 0.0034 | 0.0038 | 0.0043 | 0.0047 | 0.0051 | 0.0056 | 0.0064 |
| 0.210          | 0.0022 | 0.0026 | 0.0031 | 0.0035 | 0.0039 | 0.0044 | 0.0048 | 0.0053 | 0.0057 | 0.0066 |
| 0.215          | 0.0022 | 0.0027 | 0.0031 | 0.0036 | 0.004  | 0.0045 | 0.0049 | 0.0054 | 0.0058 | 0.0067 |
| 0.220          | 0.0023 | 0.0028 | 0.0032 | 0.0037 | 0.0041 | 0.0046 | 0.005  | 0.0055 | 0.006  | 0.0069 |
| 0.225          | 0.0023 | 0.0028 | 0.0033 | 0.0038 | 0.0042 | 0.0047 | 0.0052 | 0.0056 | 0.0061 | 0.007  |

## 8.0 Counting Rules

Microbial identification should be performed by trained and qualified aerobiologists and microscopists:

Rules for counting are laboratory dependent. Below are suggestions for counting techniques:

1. Count every particle in each field of view.
2. For any spore that is only partially visible in a given field diameter, only count that spore as a fraction ½.
3. Count spores and identify that hyphae is present.
4. Count broken spores as ½ spores.
5. Spores that are not positively identified shall be classified as unknown.
6. When spore observation in a field diameter is excessive and an estimate is used, it shall be noted as such.