

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

LAB ID:	LABORATORY NAME:
DATE:	ASSESSOR NAME:

**AIRBORNE FIBERS BY PHASE-CONTRAST MICROSCOPY**

**Method Number:**

**SOP Number:**

**Revision Number:**

**SOP Date:**

**Personnel records observed:**

Name(s) of Permanent Employee

Name(s) of Seasonal Employee (if applicable)

**Total Number of Samples Analyzed on Annual Basis (as reported to NYSDOH for fee calculation)**

Year 1

Year 2

Year 3

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

	NELAC Reference 2016	Method Reference	Y	N	N/A	Comment	Code
<b>AIRBORNE FIBERS BY PHASE-CONTRAST LIGHT MICROSCOPY</b>							
<b>I. Analytical Method</b>							
A. An approved test method is employed. (40 CFR 763 – NIOSH 7400 A Rules, Issue 3: 29 April 2019, revised 14 June 2019)							G2104
B. Method (NIOSH 7400 A Rules, Issue 3: 29 April 2019, revised 14 June 2019) is readily available to analyst.							G2106
C. Each analyst completed a NIOSH 582 course or equivalent. (Note: This applies to seasonal employees, too.)							G2108
<b>II. Sample Collection</b>							
A. The samples are collected using the following: (This pertains to clearance samples.)							
1. 25-mm cassette with 50-mm extension cowl. (Note: not applicable to Belmouth)							G2112
2. Mixed cellulose ester (MCE) filter having a pore size of 0.45 to 1.2 µm.							G2114
3. Backup pad.							G2116
4. Sampling pump, battery or line-powered vacuum with flexible connective tubing.							G2118
5. Flow rate between 0.5 and 16 L/min.							G2241
6. Minimum sampling volume of 1000 L.							G2242
7. 22-gauge multi-stranded wire.							G2243
8. One (1) inch hose clamp to attach wire to cassette for grounding, if needed.							G2244
9. Shrink or adhesive tape or cellulose bands.							G2245
B. The cassettes are stored for at least 60 days in a retrievable fashion after results are reported.							G2119
<b>III. Equipment and Supplies</b>							
A. The number of microscopes onsite is consistent with the number of samples analyzed.							For internal use only
B. The following pieces of equipment are available:							
1. Positive phase (dark) contrast light microscope equipped with:							
a. Blue or green filter.							G2126
b. 8X to 10X eyepiece and adjustable field iris.							G2128
c. 40X to 45X phase objective (numerical aperture = 0.65 to 0.75).							G2130

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

	NELAC Reference 2016	Method Reference	Y	N	N/A	Comment	Code
d. Walton-Beckett graticule type (G22 or G24) with a 100- $\mu$ m diameter circular field (area = 0.007854 mm <sup>2</sup> ).							G2132
e. Ocular phase-centering telescope, or Bertrand lens for some microscopes.							G2134
2. Phase contrast test slide. (This was formerly known as HSE/NPL slide.)							G2136
3. Stage micrometer (0.01-mm divisions).							G2138
4. Glass slides, 25 X 75 mm, pre-cleaned and frosted end. (Note: Frosted end slides are used for orientation purposes.)							G2140
5. Cover slips, 22 X 22 mm, No. 1½ unless otherwise specified by microscope manufacturer.							G2142
6. Knife with #10 surgical, steel curved blade or scissors.							G2144
7. Forceps (tweezers).							G2146
8. Flash vaporization system for cleaning filters on glass slides if using acetone.							G2148
9. Drying oven or warming plate at 60 (+2/-5) C within a fume hood if using dimethylformamide (DMF)/glacial acetic acid or Euparal resin.							G2163
10. Reagents:							
a. Reagent grade acetone for clearance procedure.							G2158
b. Reagent grade triacetin for mounting media short term.							G2160
c. Mixture of 1.4 mL (35% volume) DMF, 0.6 mL (15% volume) reagent grade glacial acetic acid, and 2 mL (50% volume) of distilled water in a dark brown vial for clearance procedure.							G2189
d. Euparal (synthetic Canada Balsam) solution for mounting media long term (>5 years).							G2191
e. <b>The volume of reagents (acetone, triacetin, DMF/glacial acetic acid) consumed is consistent with the number of samples analyzed.</b> (Note: Amount used can vary from 50 to 300 $\mu$ L.)							For internal use only
11. Labeled micropipets or microsyringes are available:							
a. for 250 $\mu$ L of acetone.							G2154
b. for 3.0-3.5 $\mu$ L of triacetin.							G2156
c. for 20 +/- 5 $\mu$ L of DMF/acetic acid.							G2172
d. for 1 drop of Euparal solution.							G2173

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

	NELAC Reference 2016	Method Reference	Y	N	N/A	Comment	Code
12. Lacquer or nail polish if using triacetin for mounting media.							G2162
<b>IV. Sample Preparation</b>							
A. At <b>least 25%</b> of the filter is used in making the slide.							G2402
B. The slides and cover slips are checked for dust and fibers. They are cleaned if necessary.							G2404
B. An acceptable procedure is used for clearing the filter.							G2406
C. An acceptable procedure is used for mounting the media after clearance.							G2407
D. Each slide is clearly labeled with the laboratory sample ID number.							G2408
E. Slides that are kept longer than 24 hours are sealed with lacquer or nail polish.							G2409
F. The slides that are kept longer than 5 years are mounted using Euparal.							G2410
<b>V. Sample Analysis</b>							
A. The count sheet contains the following:							
1. Spaces on which to record the counts for each field of view.							G2412
2. Filter identification number.							G2414
3. Analyst's name.							G2416
4. <b>Date and time</b> of analysis.							G2418
5. Total fibers counted.							G2420
6. Total fields counted.							G2422
7. Fiber density (f/mm <sup>2</sup> )							G2426
8. Microscope used for analysis.							G2427
B. A minimum of 100 fields is counted if fiber counts are less than 100. (Minimum counting time is 15 s/field per NIOSH 7400 A Rules (Issue 2, Measurement 19, Note 1 and Issue 3, Measurement 21, Note 1).							G2166
C. A minimum of 20 fields is counted if fiber counts reach 100.							G2168
D. For new employees, and following ELAP approval of the iDOC for sample load, each analyst's continued DOC is challenged <u>quarterly</u> (i.e., four times a year) by laboratory management. (Note: Analyst's initial DOC must be approved by ELAP. Refer to Asbestos FAQ #12 on ELAP website.)							G2171

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

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E. After the first year of employment, each analyst's DOC for sample load is challenged <u>semi-annually</u> (i.e., two times a year) by laboratory management.							G2165
1. The analyst's sample load is within the volume identified on his/her DOC.							G2169
<b>VI. Calibrations</b>							
A. Upon examination with the laboratory or ELAP audit slides, the following is met:							
1. Walton-Beckett graticule found to have a diameter between 98 and 102 µm (100 +/- 2 µm).  A side note: Walton-Beckett graticule: The field diameter (D) shall be verified (or checked), to a tolerance of 100 µm ± 2 µm, with a stage micrometer upon receipt of the graticule from the manufacturer. When changes (zoom adjustment, disassembly, replacement, etc.) occur in the eyepiece-objective-reticle combination, field diameter shall be re-measured.	V1M3 1.7.1.2.3						G2174
2. The actual field area is documented and used.							G2175
3. Set 3 of the phase shift test slide is visible. (Red: 4 fully visible; Green: 5 fully visible; Yellow: 6 fully visible)							G2176
4. Set 6 and higher of the phase shift test slide is invisible. (Red: 5 partially visible; Green: 6 partially visible; Yellow: 7 partially visible)							G2178
B. The following records are kept for each analyst per microscope:							
1. <b>Daily</b> check of phase-ring alignment.							G2182
2. <b>Monthly</b> check of phase shift resolution.							G2184
2.a. Check the phase-shift detection limit of the microscope periodically for each microscopist/microscope combination		Section 10.b					n/a
2.b.. The phase-shift detection limit of the microscope shall be checked monthly or after modification or relocation using an HSE/NPL phase-contrast test slide for each analyst/microscope combination.	V1M3 1.7.1.2.2					ELAP updated from weekly to a monthly check. This is the same requirement as above (G2184).	Cite G2184.
3. <b>Monthly</b> check of Walton-Beckett graticule diameter.							G2186
3.a. Walton-Beckett graticule check - no frequency stated in the method		Section 10.c				ELAP will hold the lab to a monthly check of the diameter.	See G2186.
C. Records are kept of pre- and post-survey sampling pump calibrations.							
1. Sampling pumps are calibrated against calibrated rotameters.							G2188
a. Rotameters are calibrated <b>before initial use</b> and <b>quarterly</b> against a primary standard. (Note: Examples are spirometer, bubble burette, electronic bubble meter (Giliberator®) and primary flow calibrator (mini-BUCK™).)							G2192

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

	NELAC Reference 2016	Method Reference	Y	N	N/A	Comment	Code
b. Dry gas meters (e.g., DryCal®) calibrated <b>before initial use</b> and <b>annually</b> or <b>every 200 hours of operation</b> , whichever is more frequent, against a primary standard (e.g., spirometer).							G2194
D. The records show that all clickers used to count fibers are calibrated <b>monthly</b> to 100 counts.							G2203
<b>VII. Quality Control</b>							
A. <b>New</b> filter lots are checked for background contamination before use in the field. (Note: Manufacturer-provided QA checks are normally satisfactory.)							G2198
1. Records are kept of contamination checks (performed at a frequency of 1 in 25).							G2200
2. New filter lots are rejected when the background exceeds 5 fibers per 100 fields.							G2202
B. The average field blank is determined for each set.							G2206
1. The average field blank is based on 10% of samples ( <b>at least 2 blanks</b> ) per sample set.  A Side Note: Field blanks shall be handled in a manner representative of actual handling of associated samples in the set with a single exception that air shall not be drawn through the blank sample. A blank cassette shall be opened for approximately thirty (30) seconds at the same time other cassettes are opened just prior to analysis.	V1M3 1.7.2.1.2						G2208
2. Results are qualified on reports if the field blank average exceeds 7 fibers per 100 fields.							G2210
3. Limit of detection (LOD) is estimated to be 5.5 fibers per 100 fields or 7 fibers/mm <sup>2</sup> or an LOD calculated from its own samples determined.							G2211
4. The identity of blank filters shall be unknown to the counter until all counts have been completed.	V1M3 1.7.2.1.2						G2205
C. Reference slides:							
1. Each analyst counts a minimum of one reference slide <b>per workday</b> .							G2217
2. Each analyst counts a low-fiber (5-20 fibers/100 fields) reference slide <b>at least twice per month</b> .							G2214
3. Each analyst counts a medium-fiber (21-50 fibers/100 fields) reference slide <b>at least twice per month</b> .							G2215
4. Each analyst counts a high-fiber (51-100 fibers/100 fields) reference slide <b>at least twice per month</b> .							G2216
5. The Relative Standard Deviation has been determined for each reference slide							
a. on a <b>monthly</b> basis for inexperienced (<1 year) analysts.							G2218
b. on a <b>semi-annual</b> basis for experienced (>1 year) analysts.							G2219
c. Results from the daily reference sample analysis shall be compared to	V1M3						G2212

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

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the statistically derived acceptance limits using a <i>control chart</i> or a database.	1.7.3.2.b						
d. Inter-analyst precision <i>shall be posted</i> in each laboratory to keep the microscopists informed.  A Side Note: Intra- and inter-analyst precision may be estimated from blind recounts on reference samples.	V1M3 1.7.3.2.b						G2213
6. At least 10% of all filters are re-labeled (blind recounts) by a person other than the analyst and submitted to the analyst for recounting. (Note: 20% is acceptable for a one person lab.)							G2220
7. The appropriate Relative Standard Deviation for each analyst has been applied to recounts.							G2222
8. Recounted results are rejected if the difference exceeds control limits. (See calculation below.)							G2224
9. Recount results are recorded as individual fiber counts in the 100-field count sheets.							G2225
10. A person other than the analyst re-labels slides prior to the second count.							G2227
11. <i>Inter-Laboratory Precision</i> . Each laboratory analyzing air samples for compliance determination shall implement an inter-laboratory quality assurance program that includes participation of <i>at least two (2) other independent laboratories</i> . Each laboratory shall submit slides typical of its own workload for use in this program. The round robin shall be designed, and results <i>analyzed using appropriate statistical methodology</i> .	VM3 1.7.3.2.a		X			All labs meet this requirement by participation in a NYS DOH PT study or other round robin studies such as AIHA.	n/a
<b>VIII. Reports</b>							
A. Reports include fibers/mm <sup>2</sup> and fibers/cm <sup>3</sup> ?							G2228
B. Qualifying statements are appended to airborne concentrations (fibers/cm <sup>3</sup> ) when the laboratory did not have control over sample collection.							G2230
C. The report to the client includes intra-laboratory RSD from reference slides appropriate for the set of sample concentrations reported? (Note: This RSD represents the analyst's measurement of uncertainty. Lower RSD → more accurate, higher consistency)							G2232
D. If more than one microscope is available at the lab, the laboratory identified the scope used on reports? (See count sheet requirements on page 3.)						This is optional.	SGST
E. Fiber counts above 1300 fibers/mm <sup>2</sup> and fiber counts from samples with >50% of the filter area covered with particulate shall be reported as <i>"uncountable"</i> or <i>"probably biased"</i> .	V1M3 1.7.7.2.3						G2233
F. Other fiber counts outside the 100-1300 fibers/mm <sup>2</sup> range shall be reported as having <i>"greater than optimal variability"</i> and as being <i>"probably biased"</i> .	V1M3 1.7.7.2.3						G2234
<b>IX. ELAP Consultant Audit Slide</b> Analyst needs to complete lab's count sheet for ELAP's on-site assessment records. Analysts may be challenged with at least 1 audit slide. The Pang slide is optional.							

NYSDOH Environmental Laboratory Approval Program – PCM Checklist

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A. Is at least one radial line from filter center to the outer edge of filter counted?							G2236
B. When counting each graticule field, does the analyst continuously scan a range of focal planes by moving the fine focus knob up and down?							G2238
C. Is the calculated fiber density within the acceptable range?							G2240

**Additional Notes:**

**REMINDERS:**

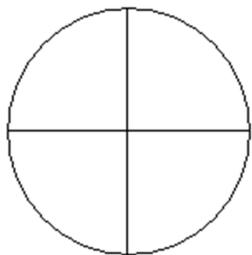
1. Assessors must use the “PCM Grid Slide Analysis Sheet for Pang Slide” below.
2. For the audit slide, have the laboratory record its results on their bench sheet.

**PCM Grid Slide Analysis Sheet for Pang Slide**

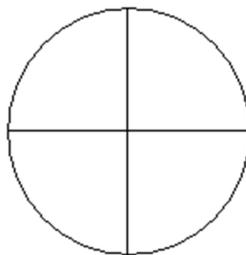
**Grid** \_\_\_\_\_ **Column** \_\_\_\_\_

**ANALYST’s Name/Initials** \_\_\_\_\_ **ASSESSOR’s Name/Initials** \_\_\_\_\_

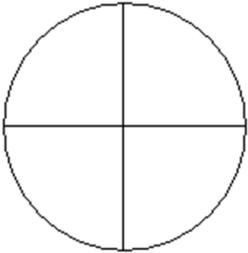
**Row 1/6**



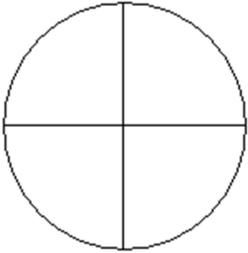
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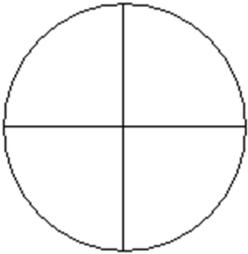
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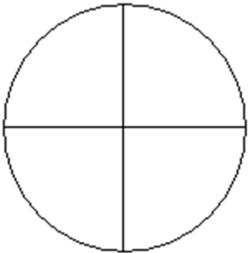
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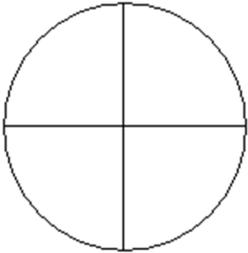
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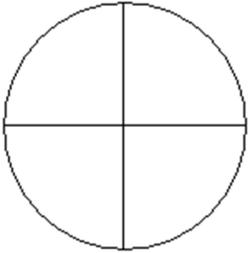
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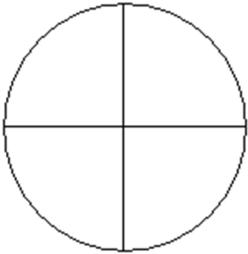
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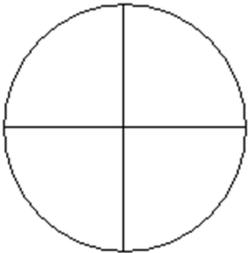
**Row 3/8**



**Row 4/9**



**Row 5/10**



**Other Useful Information (Calculations/Equations/Factors):**

Graticule Field Area ( $A_f$  as  $\text{mm}^2$ ):

$$A_f = \pi \cdot r^2$$

Where  $r = \frac{1}{2}$  the diameter ( $d$ ) (which varies between 98 to 102  $\mu\text{m}$ ) and therefore  $r$  varies between 49 to 51  $\mu\text{m}$ .

Conversion factor for  $\mu\text{m}$  to  $\text{mm}$ : 1/1000000

d ( $\mu\text{m}$ )	r ( $\mu\text{m}$ )	$A_f$ ( $\text{mm}^2$ )
98	49	0.007543
99	49.5	0.007698
100	50	0.007854
101	50.5	0.008012
102	51	0.008171

Blind Recounts:

Difference between the square roots of 2 counts (fiber/ $\text{mm}^2$ ) cannot exceed  $2.77 (X)S_r$

where  $X$  = average of the square roots of 2 counts (fiber/ $\text{mm}^2$ ),  $S_r = S_r/2$ , and  $S_r$  = intracounter relative standard deviation for the appropriate count range (fibers).

Fiber Density ( $E$  as fibers/ $\text{mm}^2$ )

$$E = \frac{(F/n_f - B/n_b)}{A_f}$$

Where  $F/n_f$  is average fiber count per graticule field,  $B/n_b$  is mean field blank per graticule field, and  $A_f$  is the graticule field area.

Concentration ( $C$  as fibers/ $\text{cm}^3$ )

$$C = \frac{(E) \cdot (A_c)}{v \cdot 10^3}$$

Where  $v$  is the air volume (liters),  $E$  is the fiber density (fibers/ $\text{mm}^2$ ), and  $A_c$  is the effective collection area of the filter (approx. 385  $\text{mm}^2$  for a 25 mm diameter filter).

Volume of Acetone Used

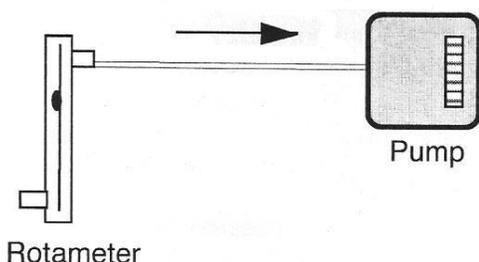
NIOSH 7400 A Rules, Issue 2, Sample Preparation 9.b., and Issue 3, A. Acetone Clearance Procedure, A2 state: "Immediately place tip of micropipette containing ca. 250  $\mu\text{l}$  acetone (use the minimum volume needed to consistently clear the filter sections) into the inlet port ..."

Example: A lab with one analyst analyzes 400 samples per week. The analyst works 54 weeks per year and does not work overtime. The analyst uses 250  $\mu\text{l}$  of acetone to vaporize the filter. How much acetone (in L) is consumed in one year for analysis purposes only? This does not include the volume of acetone lost to spillage or for cleaning utensils.

Solution:

$$(400 \text{ samples/wk}) \cdot (52 \text{ wks/yr}) \cdot (250 \text{ uL/sample}) \cdot (1\text{L}/1000000\text{uL}) = 5.2\text{L/yr}$$

Calibration of Sampling Pump Using a Rotameter



Calibration of Rotameter Using a Bubble Burette

