

Analytical Method

Fiber Count

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ANALYTICAL
METHOD

MA-243



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REVISION LIST

PAGE	DESCRIPTION OF CHANGE
All	First release of the English version of the method. This corresponds to a revised version in French.
Changes related to the previous French version:	
All	The method number no longer includes its version number. Thus, the number changes from 243-1 to 243.
2	Change in the definition of “respirable asbestos fibre”. This definition consists of the withdrawal of the criterion on the asbestos fibers diameter that shall be less than 3 μm . According to Decree 644-2022 published in the Gazette officielle du Québec on April 13, 2022, this change to Schedule I of the <i>Quebec Occupational Health and Safety Regulation</i> (RSST, RLRQ, c. S-2.1, r. 13) comes into force on October 28, 2022.
All	Some corrections and reformulations.

SUBSTANCES	CAS	STANDARDS ¹ (TWAEV ²)
Amiante – All forms	[1332-21-4]	0,1 fibre/cm ³
Actinolite	[12172-67-7]	0,1 fibre/cm ³
Amosite	[12172-73-5]	0,1 fibre/cm ³
Anthophyllite	[77536-67-5]	0,1 fibre/cm ³
Chrysotile	[12001-29-5]	0,1 fibre/cm ³
Crocidolite	[12001-28-4]	0,1 fibre/cm ³
Tremolite	[14567-73-8]	0,1 fibre/cm ³

¹ Regulation respecting occupational health and safety (Quebec)

² TWAEV Time Weighted Average Exposure Value

I. PRINCIPLE

1. A known volume of air is aspirated across a mixed cellulose ester filter (MCE) to collect the fibers according to the method described in the sampling guide (Drolet & Beauchamp, 2012) and in this method (Appendix 1).
2. The filters are cleared and maintained in a refractive index environment less than or equal to 1.46 favouring fiber observation.
3. Fiber count is performed using an optical microscope with a phase contrast condenser with a magnification of approximately 400X.
4. Regarding the *Regulation respecting occupational health and safety* (c. S-2.1, r. 13), the definition of "respirable asbestos fibre" in section 1 is: "asbestos fiber having a ratio of length to diameter of more than 3:1; only fibers longer than 5 µm must be taken into account for measurement purposes".

The term «asbestos» designates the fibrous forms of the mineral silicates belonging to metamorphic rocks of the serpentine group, namely chrysotile (white asbestos); and the amphibole group, namely actinolite, amosite (brown asbestos, cummingtonite-grunerite), anthophyllite, crocidolite (blue asbestos, riebeckite), tremolite ; or any mixture containing one or more of these minerals.

5. This counting method applies also to counts of fibers other than asbestos whose refractive index is compatible with the mounting solution.

II. APPLICABILITY

The method's field of application corresponds to densities varying from 100 to 1,300 fibers/mm². It is a function of volume sampled and the area of the counting field. Fiber densities from 25 to 100 fibers/mm², which are less than the optimum densities, can be taken into consideration to evaluate the exposure of a worker; however, the coefficient of variation of the method is not known at these densities.

III. LIMITATIONS AND INTERFERENCES

1. Sensitivity

Fibers whose diameter is less than 0.25 µm are not detected by the method (Rooker et al., 1982). The optimum upper limit can be increased by using a shorter sampling time or by decreasing the flow rate, whereas the lower limit of the field of application can be lowered by increasing the sampling volume.

2. Interferences

Any other airborne fiber can interfere if it has the geometrical count criteria described in section 1.4. Furthermore, chain-like particles can be confused with fibers. High concentrations of non-fibrous particles can obscure fibers in the field of view and increase the method's lower limit of application.

IV. PRECISION AND ACCURACY

1. Coefficient of variation

A coefficient of variation remains to be determined with representative workplace samples.

2. Quality control

2.1 Mixed cellulose ester filters

a. Filter verification

Quality control of mixed cellulose ester filters consists of checking each new lot of filters before it is used.

When a new lot of three to ten boxes is received, two boxes are checked. For a lot of ten to fifteen boxes, three boxes are checked. Take four filters per box of one hundred, or one per package of twenty-five. In each of the packages in a box, take the filter from a different section, namely from the first fourth, second fourth, etc., respectively.

Mount the filters according to the method described in section 7.1 of this document and count according to the criteria mentioned in section 7.2. These are considered as the laboratory blanks. Discard the lot of filters if the mean is greater or equal to 5 fibers per 100 graticule fields.

If one package out of four is not acceptable, again take one filter per package and check them. Again, if one filter out of four is not acceptable, the box is rejected. In the case of a lot of three to ten boxes where two boxes undergo quality control, if one box out of two is discarded, check a third one. Another discard leads to discarding the lot. In the case of a lot of eleven to fifteen boxes where three boxes undergo quality control, if one box out of three is rejected, then the fourth one is subjected to quality control; discarding it leads to discard of the entire lot.

b. Field blanks

Prepare and count the field blanks (see appendix 1) with the field samples. Note the results of each field blank. Calculate the average of the counts performed on the field blanks and subtract this value from each sample count.

Note 1: The identity of the blanks must remain unknown to the counter until the counts have been completed.

Note 2: If a field blank gives a result above 7 fibers/100 graticule fields, note a possible contamination of the samples.

2.2 Intralaboratory quality control program for fibers counters

a. Document the precision of each laboratory counter by repetitive slide counts.

- 1) Maintain a series of reference slides to be used on a daily basis as part of the laboratory's quality assurance program. These slides should consist of filter preparations including a range of densities and levels of dust from various sources including samples generated in the laboratory and others sampled in the field. The person responsible for quality assurance must keep the reference slides and supply each counter with a minimum of one reference slide per workday. He should periodically change the labels on the reference slides so that the counters do not become familiar with the samples.
- 2) Without knowing the number of the sample, repeat the count on the reference slides and estimate the intralaboratory and intercounter coefficient of variation, S_r . Obtain separate values of coefficients of variation for each matrix of samples analyzed in each of the following fields: 5 to 20 fibers in 100 graticule fields, 21 to 50 fibers in 100 graticule fields and 51 to 100 fibers in 100 graticule fields. Keep quality control graphs for each of these data.

b. Have random counts performed by the same counter on 10% of the filters counted (the slides are reidentified by a person other than the counter). Use the following calculation to determine whether a pair of counts by the same counter on one filter should be rejected because of possible bias: discard the sample if the difference between two counts exceeds $2.77 \cdot (X) \cdot S_r$, where X = the average of the two counts and S_r = the intracounter relative standard deviation.

Note: If a pair of results is rejected by this test, recount the samples in this analytical series and check the new results against the first results. Discard all rejected paired counts. It is not necessary to use these statistics on blanks.

c. Enroll each new counter in a training course that compares the performance of counters on a variety of samples using this procedure.

2.3 Interlaboratory quality control program

All laboratories involved in counting fibers should participate in a quality control program such as the AIHA PAT Programs and routinely exchange field samples with other laboratories to compare the performance of its counters.

The purpose of interlaboratory quality control is to evaluate the extent to which a count of one sample by a laboratory corresponds to the average count of a large number of laboratories. The discussion in appendix 2 indicates how this estimate can be achieved on the basis of measurement of interlaboratory variability as well as by demonstrating how the results of this method are related to the possible theoretical count precision and to the interlaboratory and intra-laboratory coefficients of variation measured.

V. REAGENTS

- Acetone

SPECIAL PRECAUTIONS:

Acetone is extremely flammable. Take precautions not to ignite it. Heating of acetone in volumes greater than 1 mL must be carried out in well-ventilated fume hood using a flameless, spark-free heat source.

- Triacetin (glycerol triacetate), reagent grade.

VI. EQUIPMENT

- Three-piece, 25 mm cassette with 50 mm conductive extension cowl, a mixed cellulose ester filter whose pore size is 0.8 to 1.2 microns, and cellulose support (see **Note 1**).
- Appropriate personal sampling pump (Drolet & Beauchamp, 2012).
- Multi-stranded 22 gauge wire.
- Positive phase contrast microscope, with green filter, 8X to 10X eyepiece, and a 40X to 45X phase objective (approximate total magnification of 400X) ; numerical aperture of 0.65 to 0.75.
- Glass slides, frosted-end, pre-cleaned, 25 mm x 75 mm.
- Cover slips, 22 mm x 22 mm, No. 1-1/2, unless otherwise indicated by the microscope manufacturer.
- Lacquer or nail polish.
- Knife, #10 surgical steel, curved blade.
- Tweezers.
- Heated aluminum block for clearing filters on glass slides, or equivalent (Baron & Pickford, 1986).
- Micropipettes, 5, 100 and 500 μ L.
- Walton-Beckett graticule, type G-22, with a 100 μ m diameter circular field (area = 0,00785 mm²) at the specimen plane (see **Note 2**).
- HSE/NPL phase contrast test slide, Mark II or Mark III.
- Telescope, ocular phase-ring centering.
- Micrometer with 0.01 mm divisions.

Comments :

Note 1: The use of an electrically conductive cowl reduces electrostatic effects. Ground the cowl when possible during sampling.

Note 2: The graticule is specific to each microscope. Specify the disc diameter required to fit exactly the ocular of the microscope and the diameter (mm) of the circular counting area (see appendix 3).

VII. ANALYTICAL PROTOCOL

1. Preparation of the sample

Number of preparation steps : 3

Step 1	Ensure that the glass slides and cover slips are free of dust and fibers.
Step 2	Adjust the temperature of the heated block to approximately 70°C (Baron & Pickford, 1986). (see Notes 1 and 2)
Step 3	<p>Mount part of the sample filter on a clean glass slide.</p> <ul style="list-style-type: none"> • Cut wedges representing approximately 25% of the filter surface with the curved-blade surgical knife using a rocking motion to avoid tearing it. Place the wedge, dust side up, on the slide (see Note 3) • Insert the slide with the sample into the opening at the base of the heating block. Place the tip of a syringe containing approximately 250 µL of acetone into the inlet port at the top of the heating block. Inject the acetone into the vaporization chamber with a slow steady pressure on the plunger while holding the syringe firmly in place. After clarification of the filter (3 to 5 seconds), remove the syringe and the slide. <p>CAUTION: Even if the volume of acetone used is small, use safety precautions. Work in a well-ventilated area (for example a laboratory fume hood). Take care not to ignite the acetone. Frequent and continuous use of this instrument in an unventilated area can produce explosive concentrations of acetone vapour.</p> <ul style="list-style-type: none"> • Place 1 to 2 drops of triacetin on the wedge. Carefully place a clean coverslip on the wedge at a slight angle to prevent the formation of air bubbles. (see Note 4) • Glue the edges of the cover slip to the slide using lacquer or nail polish (AIA, 1979) once the filter has cleared. (see Notes 5 and 6)

Comments :

- **Note 1:** If the hot block is not used in a fume hood, it must rest on a ceramic plate and must be isolated from any surface susceptible to heat damage.
- **Note 2:** Other mounting techniques can also be used (for example, the procedure to generate acetone in the vapor form in a laboratory fume hood, as described in NIOSH method 7400, revision of June 14, 2019)
- **Note 3:** Static electricity normally keeps the wedge on the slide.
- **Note 4:** If numerous air bubbles form or if the amount of triacetin is insufficient, the cover slip may become detached within a few hours. If an excess of triacetin remains at the edge of the filter under the cover slip, fiber migration may occur.
- **Note 5:** If clearing is slow, heat the slide on a hotplate (surface temperature 50°C) for up to 15 minutes. Heat carefully to prevent the formation of gas bubbles.
- **Note 6:** Counting can begin immediately once clearing and mounting are completed.

2. Fiber count (Sample analysis)

2.1 Place the slide on the microscope stage and bring the center of the filter under the objective lens. Focus on the filter plane. Adjust the microscope according to step 8.1 (Rooker et al., 1982).

Begin counting from the tip of the filter and progress outwards. Ensure that, as a minimum, each analysis covers one radial line from the filter center to the outer edge of the filter. Graticule fields are randomly selected by not looking in the eyepiece when advancing the mechanical stage.

2.2 Counting rules

1) Count as a fiber any fiber meeting the following two counting criteria and which lies completely in the observed field:

a) Count only fibers that are longer than 5 μm . Measure curved fibers by taking into account their curvature to estimate the total length.

b) Count only fibers having a length-to-width (diameter) ratio greater than three ($L/D > 3:1$).

2) Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of the fiber.

3) In the case where the fibers meeting rules 1 and 2 cross the graticule boundary:

a) Count as $\frac{1}{2}$ fiber, any fiber with only one end lying within the graticule area.

b) Do not count any fiber that crosses the graticule boundary more than once.

4) Do not count all other fibers.

5) Count enough graticule fields to obtain 100 fibers. Count a minimum of 20 fields even if more than 100 fibers are counted. Stop counting at 100 fields even if 100 fibers have not been counted.

2.3 When a bundle covers $\frac{1}{6}$ or more of a graticule field, reject the field and choose another. Do not report rejected fields in the total count.

2.4 When counting each graticule field, continuously scan a range of focal planes by moving the fine focus knob. This allows very fine fibers which have become embedded in the filter to be detected. Small-diameter fibers will be very faint but are an important contribution to the total count. A counting time of at least 15 seconds per field is appropriate for accurate counting.

Note: This method does not allow for differentiation of fibers based on morphology. Although some experienced counters are capable of selectively counting different types of fibers, there is at present no accepted method that ensures uniformity of judgement between laboratories. It is therefore incumbent upon all laboratories using this method to report total fiber counts. Should serious contamination by fibers other than asbestos occur in the samples, other techniques such as transmission electron microscopy and polarized light microscopy must be used to identify the fraction of asbestos fibers present in the sample (Dozer & Ashley, 2022; NIOSH 1994).

VIII. CALIBRATION

1. Adjustment of the microscope

Follow the manufacturer's instructions. At least once a day, use the telescope ocular supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric. For each microscope, keep a logbook to record the microscope cleaning, adjustment and calibration dates.

1.1 Each time that a sample is examined, do the following:

1.1.1 Adjust the light source for uniform illumination in the field of view at the condenser iris. With certain microscopes, illumination will have to be set up with bright field optics rather than phase contrast optics (see **Note 1**)

1.1.2 Focus on the particulate matter to be examined.

1.1.3 Ensure that the field iris is in focus, centered on the sample, and open only enough to completely illuminate the field of view.

1.2 Periodically check the microscope's detection limit for each analyst-microscope combination:

1.2.1 Center the HSE/NPL phase contrast slide under the phase objective.

1.2.2 Bring the series of grooved lines into focus in the graticule field. (see **Note 2**).

1.2.3 If the quality of the image deteriorates, clean the microscope optics. If the problem persists, consult the manufacturer.

2. Calibration of the Walton-Beckett graticule

Using a micrometer divided into hundredths of a millimeter, regularly check the diameter of the graticule and ensure that the diameter remains within an acceptable range of $100\ \mu\text{m} \pm 2\ \mu\text{m}$. The corresponding surface is $0,00785\ \text{mm}^2 \pm 0,000032\ \text{mm}^2$. To purchase a graticule, refer to appendix 3.

Comments:

Note 1: Use Köhler illumination if available.

Note 2: The slide contains seven blocks of grooves (approximately 20 grooves per block) in decreasing order of visibility. For asbestos counting, the microscope optics must completely resolve the grooved lines in block 3 even if they may appear somewhat faint, and the grooved lines in blocks 6 and 7 must be invisible when observed at the center of the graticule area. Blocks 4 and 5 must be partially visible and may vary slightly in visibility from one microscope to another. A microscope that cannot meet these criteria has too high or too low a resolution for fiber counting.

IX. CALCULATIONS AND REPORTING OF RESULTS

1. Fiber density

Calculate the fiber density on the filter by dividing the total fiber count per graticule field (N/n), minus the mean field blank count per graticule field (Nt/nt), by the graticule field area A , (0.00785 mm^2 for a properly calibrated Walton-Beckett graticule):

$$E = \frac{N/n - Nt/nt}{A}$$

Where

E = fiber density (**fibers/mm²**)
 N = Total fiber count for the sample
 n = Number of fields observed for the sample
 Nt = Average number of fields on the blank filter
 nt = Number of fields observed for the blank filter
 A = Graticule area

Comments:

Counts greater than 1300 fibers per mm^2 , and fiber counts from samples with more than 50% of the filter surface covered with particles, should be reported as "uncountable" or "probably biased".

2. Concentration

Calculate the concentration of fibers in the air volume sampled, using the effective collection area of the filter, (385 mm^2 for a 25 mm filter– see **Note**):

$$C = \frac{E * a}{d * t * 1000}$$

Where

C = Concentration (fibers/ cm^3)
 E = Fiber density (fibers/ mm^2)
 a = Effective collection area of the filter (mm^2) – see **Note**
 d = Flow rate (L/min)
 t = Sampling time (min)

Note: Periodically check that the value of « a » does not change.

3. Reporting of results

Report intra-laboratory and interlaboratory coefficients of variation (S_r) with each set of results.

Note: The precision depends on the total number of fibers counted. Relative standard deviation (also called coefficient of variation) is documented for counts up to 100 fibers in 100 reticular fields (Asbestos Information Association [AIA], 1983; Dozer & Ashley, 2022; Ogden, 1982; Schlecht & Shulman, 1986). The comparability of interlaboratory results is discussed in appendix 2. As a first approximation, use 213% above and 49% below count as the upper and lower confidence limit for fiber counts greater than 20 (figure 1 of appendix 2).

X. REFERENCES

- Asbestos International Association. (1979). *Airborne asbestos fiber concentrations at workplaces by light microscopy* (Method No. 1). AIA.
- Asbestos Information Association. (1983). *A study of the empirical precision of airborne asbestos concentration measurements in the workplace by the membrane filter method*. AIA.
- Baron, P. A. & Pickford, G. C. (1986). An asbestos sample filter clearing procedure. *Applied Industrial Hygiene*, 1(4), 169-171.
- Baron, P. A. & Shulman, S. A. (1987). Evaluation of the Magiscan image analyzer for asbestos fiber counting. *American Industrial Hygiene Association Journal*, 48(1), 39-46.
- Crawford, N. P., Thorpe, H. L. & Alexander, W. (1982). *A comparison of the effects of different counting rules and aspect ratios on the level and reproducibility of asbestos fiber counts. Part I: Effects on level* (Report No. 7M/82/23).
- Crawford, N. P., Thorpe, H. L. & Alexander, W. (1982). *A comparison of the effects of different counting rules and aspect ratios on the level and reproducibility of asbestos fiber counts. Part II: Effects on reproducibility* (Report No. 7M/82/24). Institute of Occupational Medicine.
- Dozer, A. & Ashley, E. (2022). Measurement and characterization of fibrous particles in workplace atmospheres. In National Institute for Occupational Safety and Health (Ed.), *NIOSH manual of analytical methods* (5^e éd.). NIOSH.
- Drolet, D. & Beauchamp, G. (2012). *Sampling guide for air contaminants in the workplace* (Method No. T-06, 8th ed.). IRSST.
- Johnston, A. M., Jones, A. D. & Vincent, J. H. (1982). The influence of external aerodynamic factors on the measurement of the airborne concentration of asbestos fibers by the membrane filter method. *Annals of Occupational Hygiene*, 25(3), 309-316.
- National Institute for Occupational Safety and Health. (1994). *Asbestos (bulk) by PLM* (Method No. 9002). NIOSH.
- National Institute for Occupational Safety and Health. (2019). *Asbestos and other fibers by PCM*. NIOSH.
- Ogden, T. L. (1982). *The reproducibility of fiber counts* (Report No. 18). HSE.
- Regulation respecting the quality of the work environment*, c. S-2.1, r. 11.
- Regulation respecting occupational health and safety*, c. S-2.1, r. 13.
- Rooker, S. J., Vaughn, N. P. & LeGuen, J. M. (1982). On the visibility of fibers by phase contrast microscopy. *American Industrial Hygiene Association Journal*, 43(7), 505-515.
- Schlecht, P. C. & Shulman, S. A. (1986). Performance of asbestos fiber counting laboratories in the NIOSH Proficiency Analytical Testing (PAT) program. *American Industrial Hygiene Association Journal*, 47(5), 259-266.

APPENDIX 1

SAMPLING

1. Adjust the flow rate of each personal sampling pump with an appropriate instrument (Drolet & Beauchamp, 2012).
2. For personal sampling, attach the sampler to the worker near his breathing zone. Remove the cover from the cowl extension and orient face down. Wrap shrink tape between the extension and the cassette to avoid air leaks.

Note: If possible, ground the cassette to eliminate any surface charge by using a wire connecting the conducting extension to a non-electrical metal fixture such as a cold water pipe or a steel beam.

3. Submit for each series of samples at least two field blanks or 10% of the total samples, whichever is greater.
4. Sample at 0.5 L/min or more (Johnston et al., 1982). Adjust the sampling flow rate, d (L/min) and the time t (min) in such a way as to obtain a fiber density, E , of 100 to 1,300 fibers/mm² on a 25 mm filter (effective collection area of 385 mm²) to obtain optimum precision.

$$t = \frac{a * E}{d * C * 1000}$$

Note: The purpose of adjusting the sampling time is to obtain optimum fiber loading on the filter. A sampling rate of 1 to 4 L/min over a period of 8 hours is appropriate in non-dusty atmospheres containing for example 0.1 fiber/mL. Dusty atmospheres require smaller sampling volumes (less or equal to 400 L) to obtain countable samples.

In such cases, take consecutive samples of short duration. Determine the average concentration value as described in *Regulation respecting the quality of the work environment* (c. S-2.1, r.11). In order to document episodic exposure, use high flow rates (7 to 16 L/min) with shorter sampling times. In relatively clean atmospheres, where the fiber concentration is less than 0.1 fiber/mL, use larger sample volumes (3000 to 10000 L) to obtain quantifiable densities.

However, take care not to overload the dust filter. If 50% or more of the surface is covered with particles, the filter may be overloaded, and thus the measured fiber concentration would be biased.

5. At the end of sampling, replace the covers and end caps.
6. Ship the samples with the conductive cowl attached in a rigid container in order to avoid jostling or damage.

Note: Do not use untreated polystyrene foam as a shipping container because electrostatic forces may result in fiber losses from the filter surface.

APPENDIX 2

INTERLABORATORY COMPARABILITY

Theoretically, the process of counting fibers distributed randomly (Poisson) on the filter surface gives an Sr that depends on the number, N, of fibers counted:

$$Sr = \frac{1}{N^{1/2}}$$

Sr is therefore 0.1 for 100 fibers and 0.32 for 10 fibers counted. The actual Sr found in a number of studies is greater than these theoretical values.

An additional component of variability comes from subjective differences between laboratories.

In a study involving 10 counters in a continuing sample exchange program, Ogden (1982) found that this intra-laboratory subjective component is approximately 0.2 and he estimated the overall Sr by the expression:

$$Sr = \frac{\sqrt{N+(0.2*N)^2}}{N}$$

Ogden found that a confidence interval of 90%, the individual intra-laboratory counts in relation to the means were +2 Sr and -1.5 Sr. In this program, one sample out of ten was a quality control sample. In the case of laboratories that are not engaged in an intensive quality assurance program, the subjective variability component can be higher.

In a study of field sample results from 46 laboratories, the Asbestos Information Association (AIA) found that the variability had both a constant component and a component that depends on the number of fibers (1983). These results gave a subjective interlaboratory component of the coefficient of variation for the field samples of about 0.45 (on the same basis as Ogden). A similar value was obtained for 12 laboratories analyzing a series of 24 samples (Baron et Shulman, 1987). This value is slightly higher than the range of Sr (0.25 to 0.42 for 1984-85) found for 80 reference laboratories in the NIOSH PAT program for laboratory-generated samples (Schlecht & Shulman, 1986).

Several factors influence the value of Sr for a given laboratory, such as the actual counting performance of a laboratory and the types of samples to be analyzed. In the absence of other information, such as interlaboratory quality assurance programs using field samples, the value of the subjective component of variability used is 0.45. It should be noted that, although based on two studies, it is a somewhat arbitrary choice. It is hoped that by using this value in the absence of additional information, laboratories will carry out the recommended interlaboratory quality assurance programs to increase their performance and reduce their Sr.

The relative standard deviations (Sr) described above apply when the mean of the population has been determined. However, it is more appropriate for laboratories to estimate a 90% confidence interval on the mean fiber count from a single sample (figure 1). These curves assume similar shapes of the count distribution for interlaboratory and intra-laboratory results (Ogden, 1982).

For example, if a sample gives a count of 24 fibers, Figure 1 indicates that in 90% of the cases, the mean interlaboratory count will fall within the range of 227% above and 52% below this value. These percentages can be applied directly to the concentrations in the air. If for example, this sample (24 fibers counted) represents a volume of 500 liters, then the measured concentration is 0.02 fibers/mL (assuming 100 fields counted, a 25 mm filter and a 0.00785 mm² counting field area). If this same sample were counted by a group of laboratories, there is a 90% probability that the mean would fall between 0.01 and 0.08 fibers/mL. These limits should be reported in any comparison of results between laboratories.

It should be noted that the S_r of 0.45 used to calculate Figure 1 is considered as an estimate for a random group of laboratories. If several laboratories belonging to a quality insurance group can show that their interlaboratory S_r is smaller, then it is more correct to use the smallest S_r . It has been found that S_r can be greater for certain types of samples, such as asbestos cement (Crawford et al., 1982).

One can see from figure 1 that the Poisson component of the variability is not very important unless the number of fibers counted is small. As a result, an acceptable approximation consists of simply using +213% and -49% as the upper and lower confidence limits of the mean for a count of 100 fibers.

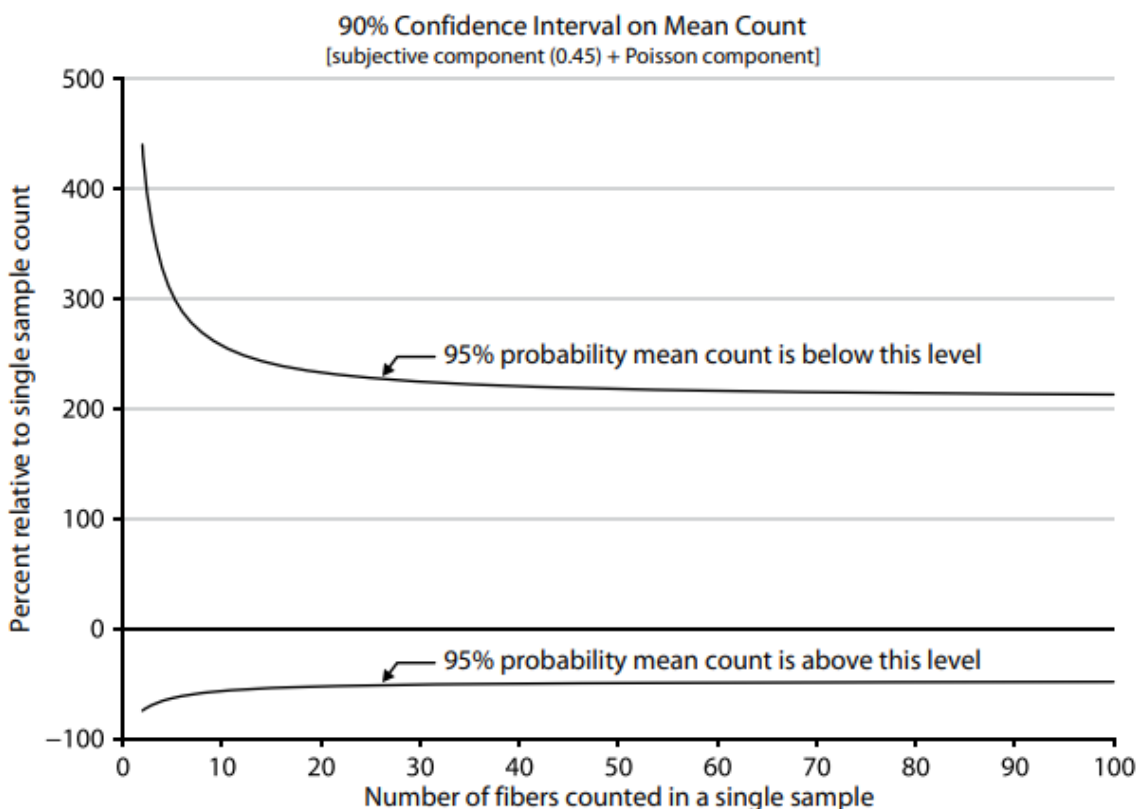


Figure 1. Interlaboratory Precision

APPENDIX 3

CALIBRATION OF THE WALTON-BECKETT GRATICULE

Before ordering a Walton-Beckett graticule, the following calibration must be carried out in order to obtain a counting area (D) of 100 µm in diameter at the image plane. The diameter d_c (mm) of the circular counting area and the diameter of the disc must be specified when ordering the graticule.

- 1) Insert any available graticule in the eyepiece and focus so that the graticule lines are sharp and clear.
- 2) Set the appropriate interpupillary distance and if applicable, reset the binocular head adjustment in such a way that magnification remains constant.
- 3) Install the 40x to 45x phase objective.
- 4) Place a stage micrometer on the microscope object stage and focus the microscope on the graduated lines.
- 5) Measure the length of the magnified graticule grid, L_0 (µm), using the stage micrometer.
- 6) Remove the microscope graticule and measure its actual grid length, L_a (mm). This can best be accomplished using a stage equipped with verniers.
- 7) Calculate the diameter of the circle, d_c (mm), for the Walton-Beckett graticule:

$$d_c = \frac{L_a * D}{L_0}$$

For example: if $L_0 = 112$ µm, $L_a = 4,5$ mm and $D = 100$ µm, then $d_c = 4,02$ mm.

Upon receipt of the graticule from the manufacturer, check the field diameter, D (acceptable range of 100 µm ± 2 µm) with the micrometer. Determine the field area (acceptable range of 0.00785 mm² ± 0.00032 mm²).

APPENDIX 4

EXAMPLES OF COUNTING RULES

Figure 2 shows a Walton-Beckett graticule as it is seen through a microscope. Even if the graticule incorporates the 3:1 aspect, the counting rules will be discussed since they apply to the fibers identified in figure 2.

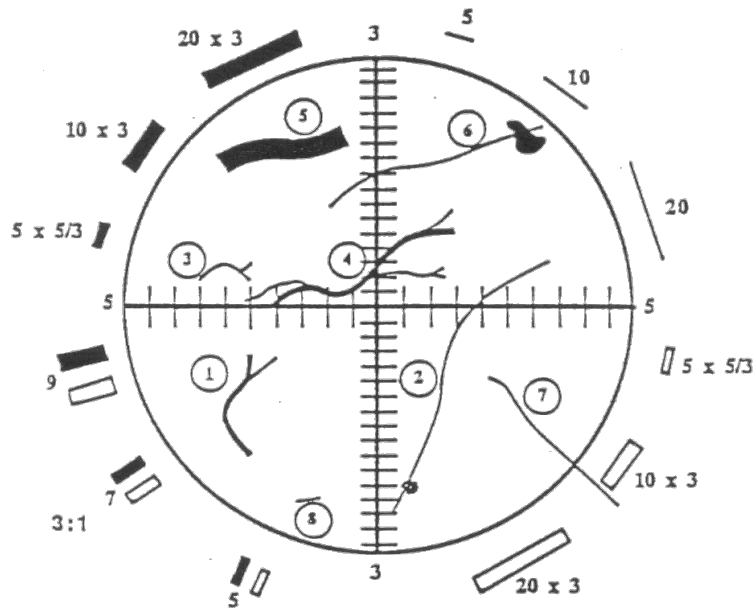


Figure 2: Walton-Beckett graticule with fibers

FIBER COUNT

Fiber number	Count	Discussion
1	1 fiber	The criteria do not allow for split ends ; as a result, count one fiber;
2	1 fiber	Single fiber with attached particle. The particle is treated as though it does not exist.
3	1 fiber	As for fiber 1, count one fiber because it meets the >3:1, > 5 μm in length criteria.
4	1 fiber	All fiber ends are attached to a large central fiber or bundle; as a result, count one fiber.
5	1 fiber	As for fiber 3, count one fiber because it meets the >3:1, > 5 μm in length criteria. (it counts even if the fiber has a diameter greater than 3 μm)
6	1 fiber	Ignore non-fibrous particulate matter; count this as a whole fiber.
7	$\frac{1}{2}$ fiber	Fibers that meet criteria 7.2.2.a and 7.2.2.b and that cross the graticule boundary are counted as half fibers provided the fiber does not cross the graticule boundary more than once. In such a case, the fiber is not counted, regardless of the number of ends that lie within the graticule area.
8	0 fiber	The fiber is shorter than 5 μm .