ASBESTOS and OTHER FIBERS by PCM 7400

Various MW: Various CAS: Various RTECS: Various

METHOD: 7400, Issue 2 **EVALUATION: FULL** **Issue 1: Rev. 3 on 15 May 1989**
Issue 2: 15 August 1994

OSHA : 0.1 asbestos fiber (> 5 µm long)/cc; 1 f/cc/30 min excursion; carcinogen
MSHA: 2 asbestos fibers/cc
NIOSH: 0.1 f/cc (fibers > 5 µm long)/400 L; carcinogen
ACGIH: 0.2 crocidolite; 0.5 amosite; 2 chrysotile and other asbestos, fibers/cc; carcinogen

PROPERTIES: solid, fibrous, crystalline, anisotropic

SYNONYMS [CAS #]: actinolite **[77536-66-4]** or ferroactinolite **[15669-07-5]**; amosite **[12172-73-5]**; anthophyllite **[77536-67-5]**; chrysotile **[12001-29-5]**; serpentine **[18786-24-8]**; crocidolite **[12001-28-4]**; tremolite **[77536-68-6]**; amphibole asbestos **[1332-21-4]**; refractory ceramic fibers **[142844-00-6]**; fibrous glass.

SAMPLING

SAMPLER: FILTER (0.45- to 1.2-µm cellulose ester membrane, 25-mm; conductive cowl on cassette)

FLOW RATE*: 0.5 to 16 L/min

VOL-MIN*: 400 L @ 0.1 fiber/cc
-MAX*: (step 4, sampling)
*Adjust to give 100 to 1300 fiber/mm^2

SHIPMENT: routine (pack to reduce shock)

SAMPLE STABILITY: stable

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: 80 to 100 fibers counted

BIAS: See EVALUATION OF METHOD

OVERALL PRECISION ($\hat{S}_{rT}$): 0.115 to 0.13 [1]

ACCURACY: See EVALUATION OF METHOD

MEASUREMENT

TECHNIQUE: LIGHT MICROSCOPY, PHASE CONTRAST

ANALYTE: fibers (manual count)

SAMPLE PREPARATION: acetone - collapse/triacetin - immersion

COUNTING RULES: described in previous version of this method as "A" rules [1,3]

EQUIPMENT:
1. positive phase-contrast microscope
2. Walton-Beckett graticule (100-µm field of view) Type G-22
3. phase-shift test slide (HSE/NPL)

CALIBRATION: HSE/NPL test slide

RANGE: 100 to 1300 fibers/mm^2 filter area

ESTIMATED LOD: 7 fibers/mm^2 filter area

PRECISION ($\bar{S}_r$): 0.10 to 0.12 [1]; see EVALUATION OF METHOD

APPLICABILITY: The quantitative working range is 0.04 to 0.5 fiber/cc for a 1000-L air sample. The LOD depends on sample volume and quantity of interfering dust, and is <0.01 fiber/cc for atmospheres free of interferences. The method gives an index of airborne fibers. It is primarily used for estimating asbestos concentrations, though PCM does not differentiate between asbestos and other fibers. Use this method in conjunction with electron microscopy (e.g., Method 7402) for assistance in identification of fibers. Fibers < ca. 0.25 µm diameter will not be detected by this method [4]. This method may be used for other materials such as fibrous glass by using alternate counting rules (see Appendix C).

INTERFERENCES: If the method is used to detect a specific type of fiber, any other airborne fiber may interfere since all particles meeting the counting criteria are counted. Chain-like particles may appear fibrous. High levels of non-fibrous dust particles may obscure fibers in the field of view and increase the detection limit.

OTHER METHODS: This revision replaces Method 7400, Revision #3 (date 5/15/89).

REAGENTS:

1. Acetone,* reagent grade.
2. Triacetin (glycerol triacetate), reagent grade.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: field monitor, 25-mm, three-piece cassette with ca. 50-mm electrically conductive extension cowl and cellulose ester filter, 0.45- to 1.2-µm pore size, and backup pad.

   NOTE 1: Analyze representative filters for fiber background before use to check for clarity and background. Discard the filter lot if mean is ≥5 fibers per 100 graticule fields. These are defined as laboratory blanks. Manufacturer-provided quality assurance checks on filter blanks are normally adequate as long as field blanks are analyzed as described below.

   NOTE 2: The electrically conductive extension cowl reduces electrostatic effects. Ground the cowl when possible during sampling.

   NOTE 3: Use 0.8-µm pore size filters for personal sampling. The 0.45-µm filters are recommended for sampling when performing TEM analysis on the same samples. However, their higher pressure drop precludes their use with personal sampling pumps.

   NOTE 4: Other cassettes have been proposed that exhibit improved uniformity of fiber deposit on the filter surface, e.g., bellmouthed sampler (Envirometrics, Charleston, SC). These may be used if shown to give measured concentrations equivalent to sampler indicated above for the application.
2. Personal sampling pump, battery or line-powered vacuum, of sufficient capacity to meet flow-rate requirements (see step 4 for flow rate), with flexible connecting tubing.
3. Wire, multi-stranded, 22-gauge; 1", hose clamp to attach wire to cassette.
4. Tape, shrink- or adhesive-.
5. Slides, glass, frosted-end, pre-cleaned, 25 x 75-mm.
6. Cover slips, 22- x 22-mm, No. 1-1/2, unless otherwise specified by microscope manufacturer.
7. Lacquer or nail polish.
8. Knife, #10 surgical steel, curved blade.
9. Tweezers.

EQUIPMENT:

10. Acetone flash vaporization system for clearing filters on glass slides (see ref. [5] for specifications or see manufacturer's instructions for equivalent devices).
11. Micropipets or syringes, 5-µL and 100- to 500-µL.
12. Microscope, positive phase (dark) contrast, with green or blue filter, adjustable field iris, 8 to 10X eyepiece, and 40 to 45X phase objective (total magnification ca. 400X); numerical aperture = 0.65 to 0.75.
13. Graticule, Walton-Beckett type with 100-µm diameter circular field (area = 0.00785 mm2) at the specimen plane (Type G-22). Available from Optometrics USA, P.O. Box 699, Ayer, MA 01432 [phone (508)-772-1700], and McCrone Accessories and Components, 850 Pasquinelli Drive, Westmont, IL 60559 [phone (312) 887-7100].
    NOTE: The graticule is custom-made for each microscope. (see APPENDIX A for the custom-ordering procedure).
14. HSE/NPL phase contrast test slide, Mark II. Available from Optometrics USA (address above).
15. Telescope, ocular phase-ring centering.
16. Stage micrometer (0.01-mm divisions).

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

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. To reduce contamination and to hold the cassette tightly together, seal the crease between the cassette base and the cowl with a shrink band or light colored adhesive tape. For personal sampling, fasten the (uncapped) open-face cassette to the worker's lapel. The open face should be oriented downward.
   NOTE: The cowl should be electrically grounded during area sampling, especially under conditions of low relative humidity. Use a hose clamp to secure one end of the wire (Equipment, Item 3) to the monitor's cowl. Connect the other end to an earth ground (i.e., cold water pipe).
3. Submit at least two field blanks (or 10% of the total samples, whichever is greater) for each set of samples. Handle field blanks in a manner representative of actual handling of associated samples in the set. Open field blank cassettes at the same time as other cassettes just prior to sampling. Store top covers and cassettes in a clean area (e.g., a closed bag or box) with the top covers from the sampling cassettes during the sampling period.
4. Sample at 0.5 L/min or greater [6]. Adjust sampling flow rate, Q (L/min), and time, t (min), to produce a fiber density, E, of 100 to 1300 fibers/mm^2 ($3.85 \cdot 10^4$ to $5 \cdot 10^5$ fibers per 25-mm filter with effective collection area A_c= 385 mm^2) for optimum accuracy. These variables are related to the action level (one-half the current standard), L (fibers/cc), of the fibrous aerosol being sampled by:

$$t = \frac{A_c \cdot E}{Q \cdot L \cdot 10^3}, \text{ min.}$$

NOTE 1: The purpose of adjusting sampling times is to obtain optimum fiber loading on the filter. The collection efficiency does not appear to be a function of flow rate in the range of 0.5 to 16 L/min for asbestos fibers [7]. Relatively large diameter fibers (>3 µm) may exhibit significant aspiration loss and inlet deposition. A sampling rate of 1 to 4 L/min for 8 h is appropriate in atmospheres containing ca. 0.1 fiber/cc in the absence of significant amounts of non-asbestos dust. Dusty atmospheres require smaller sample volumes (≤400 L) to obtain countable samples. In such cases take short, consecutive samples and average the results over the total collection time. For documenting episodic exposures, use high flow rates (7 to 16 L/min) over shorter sampling times. In relatively clean atmospheres, where targeted fiber concentrations are much less than 0.1 fiber/cc, use larger sample volumes (3000 to 10000 L) to achieve quantifiable loadings. Take care, however, not to overload the filter with background dust. If ≥ 50% of the filter surface is covered with particles, the filter may be too overloaded to count and will bias the measured fiber concentration.

NOTE 2: OSHA regulations specify a minimum sampling volume of 48 L for an excursion measurement, and a maximum sampling rate of 2.5 L/min [3].

5. At the end of sampling, replace top cover and end plugs.
6. Ship samples with conductive cowl attached in a rigid container with packing material to prevent jostling or damage.
   NOTE: Do not use untreated polystyrene foam in shipping container because electrostatic forces may cause fiber loss from sample filter.

SAMPLE PREPARATION:

NOTE 1: The object is to produce samples with a smooth (non-grainy) background in a medium with refractive index ≤1.46. This method collapses the filter for easier focusing and produces permanent (1 - 10 years) mounts which are useful for quality control and interlaboratory comparison. The aluminum "hot block" or similar flash vaporization techniques may be used outside the laboratory [2]. Other mounting techniques meeting the above criteria may also be used (e.g., the laboratory fume hood procedure for generating acetone vapor as described in Method 7400 - revision of 5/15/85, or the non-permanent field mounting technique used in P&CAM 239 [3,7,8,9]). Unless the effective filtration area is known, determine the area and record the information referenced against the sample ID number [1,9,10,11].

NOTE 2: Excessive water in the acetone may slow the clearing of the filter, causing material to be washed off the surface of the filter. Also, filters that have been exposed to high humidities prior to clearing may have a grainy background.

7. Ensure that the glass slides and cover slips are free of dust and fibers.
8. Adjust the rheostat to heat the "hot block" to ca. 70 °C [2].
   NOTE: If the "hot block" is not used in a fume hood, it must rest on a ceramic plate and be isolated from any surface susceptible to heat damage.
9. Mount a wedge cut from the sample filter on a clean glass slide.
   a. Cut wedges of ca. 25% of the filter area with a curved-blade surgical steel knife using a rocking motion to prevent tearing. Place wedge, dust side up, on slide.
      NOTE: Static electricity will usually keep the wedge on the slide.

b. Insert slide with wedge into the receiving slot at base of "hot block". Immediately place tip of a micropipet containing ca. 250 µL acetone (use the minimum volume needed to consistently clear the filter sections) into the inlet port of the PTFE cap on top of the "hot block" and inject the acetone into the vaporization chamber with a slow, steady pressure on the plunger button while holding pipet firmly in place. After waiting 3 to 5 sec for the filter to clear, remove pipet and slide from their ports.

CAUTION: Although the volume of acetone used is small, use safety precautions. Work in a well-ventilated area (e.g., laboratory fume hood). Take care not to ignite the acetone. Continuous use of this device in an unventilated space may produce explosive acetone vapor concentrations.

c. Using the 5-µL micropipet, immediately place 3.0 to 3.5 µL triacetin on the wedge. Gently lower a clean cover slip onto the wedge at a slight angle to reduce bubble formation. Avoid excess pressure and movement of the cover glass.

NOTE: If too many bubbles form or the amount of triacetin is insufficient, the cover slip may become detached within a few hours. If excessive triacetin remains at the edge of the filter under the cover slip, fiber migration may occur.

d. Mark the outline of the filter segment with a glass marking pen to aid in microscopic evaluation.

e. Glue the edges of the cover slip to the slide using lacquer or nail polish [12]. Counting may proceed immediately after clearing and mounting are completed.

NOTE: If clearing is slow, warm the slide on a hotplate (surface temperature 50 °C) for up to 15 min to hasten clearing. Heat carefully to prevent gas bubble formation.

CALIBRATION AND QUALITY CONTROL:

10. Microscope adjustments. Follow the manufacturers instructions. At least once daily use the telescope ocular (or Bertrand lens, for some microscopes) supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric. With each microscope, keep a logbook in which to record the dates of microscope cleanings and major servicing.

    a. Each time a sample is examined, do the following:

        (1) Adjust the light source for even illumination across the field of view at the condenser iris. Use Kohler illumination, if available. With some microscopes, the illumination may have to be set up with bright field optics rather than phase contract optics.

        (2) Focus on the particulate material to be examined.

        (3) Make sure that the field iris is in focus, centered on the sample, and open only enough to fully illuminate the field of view.

    b. Check the phase-shift detection limit of the microscope periodically for each analyst/microscope combination:

        (1) Center the HSE/NPL phase-contrast test slide under the phase objective.

        (2) Bring the blocks of grooved lines into focus in the graticule area.

        NOTE: The slide contains seven blocks of grooves (ca. 20 grooves per block) in descending order of visibility. For asbestos counting the microscope optics must completely resolve the grooved lines in block 3 although they may appear somewhat faint, and the grooved lines in blocks 6 and 7 must be invisible when centered in the graticule area. Blocks 4 and 5 must be at least partially visible but may vary slightly in visibility between microscopes. A microscope which fails to meet these requirements has resolution either too low or too high for fiber counting.

        (3) If image quality deteriorates, clean the microscope optics. If the problem persists, consult the microscope manufacturer.

11. Document the laboratory's precision for each counter for replicate fiber counts.

    a. Maintain as part of the laboratory quality assurance program a set of reference slides to be used on a daily basis [13]. These slides should consist of filter preparations including a range of loadings and background dust levels from a variety of sources including both field and reference samples (e.g., PAT, AAR, commercial samples). The Quality Assurance Officer

should maintain custody of the reference slides and should supply each counter with a minimum of one reference slide per workday. Change the labels on the reference slides periodically so that the counter does not become familiar with the samples.

b. From blind repeat counts on reference slides, estimate the laboratory intra- and intercounter precision. Obtain separate values of relative standard deviation (S_r) for each sample matrix analyzed in each of the following ranges: 5 to 20 fibers in 100 graticule fields, >20 to 50 fibers in 100 graticule fields, and >50 to 100 fibers in 100 graticule fields. Maintain control charts for each of these data files.

NOTE: Certain sample matrices (e.g., asbestos cement) have been shown to give poor precision [9]

12. Prepare and count field blanks along with the field samples. Report counts on each field blank.

NOTE 1: The identity of blank filters should be unknown to the counter until all counts have been completed.

NOTE 2: If a field blank yields greater than 7 fibers per 100 graticule fields, report possible contamination of the samples.

13. Perform blind recounts by the same counter on 10% of filters counted (slides relabeled by a person other than the counter). Use the following test to determine whether a pair of counts by the same counter on the same filter should be rejected because of possible bias: Discard the sample if the absolute value of the difference between the square roots of the two counts (in fiber/mm^2) exceeds 2.77 (X)S_r', where X = average of the square roots of the two fiber counts (in fiber/mm^2) and $S_r' = \frac{S_r}{2}$, where S_r is the intracounter relative standard deviation for the appropriate count range (in fibers) determined in step 11. For more complete discussions see reference [13].

NOTE 1: Since fiber counting is the measurement of randomly placed fibers which may be described by a Poisson distribution, a square root transformation of the fiber count data will result in approximately normally distributed data [13].

NOTE 2: If a pair of counts is rejected by this test, recount the remaining samples in the set and test the new counts against the first counts. Discard all rejected paired counts. It is not necessary to use this statistic on blank counts.

14. The analyst is a critical part of this analytical procedure. Care must be taken to provide a non-stressful and comfortable environment for fiber counting. An ergonomically designed chair should be used, with the microscope eyepiece situated at a comfortable height for viewing. External lighting should be set at a level similar to the illumination level in the microscope to reduce eye fatigue. In addition, counters should take 10-to-20 minute breaks from the microscope every one or two hours to limit fatigue [14]. During these breaks, both eye and upper back/neck exercises should be performed to relieve strain.

15. All laboratories engaged in asbestos counting should participate in a proficiency testing program such as the AIHA-NIOSH Proficiency Analytical Testing (PAT) Program for asbestos and routinely exchange field samples with other laboratories to compare performance of counters.

MEASUREMENT:

16. Center the slide on the stage of the calibrated microscope under the objective lens. Focus the microscope on the plane of the filter.

17. Adjust the microscope (Step 10).

NOTE: Calibration with the HSE/NPL test slide determines the minimum detectable fiber diameter (ca. 0.25 µm) [4].

18. Counting rules: (same as P&CAM 239 rules [1,10,11]: see examples in APPENDIX B).

a. Count any fiber longer than 5 µm which lies entirely within the graticule area.

(1) Count only fibers longer than 5 µm. Measure length of curved fibers along the curve.

(2) Count only fibers with a length-to-width ratio equal to or greater than 3:1.

b. For fibers which cross the boundary of the graticule field:

(1) Count as 1/2 fiber any fiber with only one end lying within the graticule area, provided that the fiber meets the criteria of rule a above.

(2) Do not count any fiber which crosses the graticule boundary more than once.
(3) Reject and do not count all other fibers.

c. Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of a fiber.
d. Count enough graticule fields to yield 100 fibers. Count a minimum of 20 fields. Stop at 100 graticule fields regardless of count.

19. Start counting from the tip of the filter wedge and progress along a radial line to the outer edge. Shift up or down on the filter, and continue in the reverse direction. Select graticule fields randomly by looking away from the eyepiece briefly while advancing the mechanical stage. Ensure that, as a minimum, each analysis covers one radial line from the filter center to the outer edge of the filter. When an agglomerate or bubble covers ca. 1/6 or more of the graticule field, reject the graticule field and select another. Do not report rejected graticule fields in the total number counted.

NOTE 1: When counting a graticule field, continuously scan a range of focal planes by moving the fine focus knob to detect very fine fibers which have become embedded in the filter. The small-diameter fibers will be very faint but are an important contribution to the total count. A minimum counting time of 15 seconds per field is appropriate for accurate counting.

NOTE 2: This method does not allow for differentiation of fibers based on morphology. Although some experienced counters are capable of selectively counting only fibers which appear to be asbestiform, there is presently no accepted method for ensuring uniformity of judgment between laboratories. It is, therefore, incumbent upon all laboratories using this method to report total fiber counts. If serious contamination from non-asbestos fibers occurs in samples, other techniques such as transmission electron microscopy must be used to identify the asbestos fiber fraction present in the sample (see NIOSH Method 7402). In some cases (i.e., for fibers with diameters >1 µm), polarized light microscopy (as in NIOSH Method 7403) may be used to identify and eliminate interfering non-crystalline fibers [15].

NOTE 3: Do not count at edges where filter was cut. Move in at least 1 mm from the edge.

NOTE 4: Under certain conditions, electrostatic charge may affect the sampling of fibers. These electrostatic effects are most likely to occur when the relative humidity is low (below 20%), and when sampling is performed near the source of aerosol. The result is that deposition of fibers on the filter is reduced, especially near the edge of the filter. If such a pattern is noted during fiber counting, choose fields as close to the center of the filter as possible [5].

NOTE 5: Counts are to be recorded on a data sheet that provides, as a minimum, spaces on which to record the counts for each field, filter identification number, analyst's name, date, total fibers counted, total fields counted, average count, fiber density, and commentary. Average count is calculated by dividing the total fiber count by the number of fields observed. Fiber density (fibers/mm^2) is defined as the average count (fibers/field) divided by the field (graticule) area (mm^2/field).

CALCULATIONS AND REPORTING OF RESULTS

20. Calculate and report fiber density on the filter, E (fibers/mm^2), by dividing the average fiber count per graticule field, F/n_f, minus the mean field blank count per graticule field, B/n_b, by the graticule field area, A_f (approx. 0.00785 mm^2):

$$E = \frac{\left(\frac{F}{n_f} - \frac{B}{n_b}\right)}{A_f}, \text{ fibers/mm}^2.$$

NOTE: Fiber counts above 1300 fibers/mm^2 and fiber counts from samples with >50% of filter area covered with particulate should be reported as "uncountable" or "probably biased." Other fiber counts outside the 100-1300 fiber/mm^2 range should be reported as having "greater than optimal variability" and as being "probably biased."

21. Calculate and report the concentration, C (fibers/cc), of fibers in the air volume sampled, V (L), using the effective collection area of the filter, A_c (approx. 385 mm^2 for a 25-mm filter):

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

NOTE: Periodically check and adjust the value of A_c, if necessary.

22. Report intralaboratory and interlaboratory relative standard deviations (from Step 11) with each set of results.

NOTE: Precision depends on the total number of fibers counted [1,16]. Relative standard deviation is documented in references [1,15-17] for fiber counts up to 100 fibers in 100 graticule fields. Comparability of interlaboratory results is discussed below. As a first approximation, use 213% above and 49% below the count as the upper and lower confidence limits for fiber counts greater than 20 (Fig. 1).

EVALUATION OF METHOD:

A. This method is a revision of P&CAM 239 [10]. A summary of the revisions is as follows:

1. Sampling:
   The change from a 37-mm to a 25-mm filter improves sensitivity for similar air volumes. The change in flow rates allows for 2-m^3 full-shift samples to be taken, providing that the filter is not overloaded with non-fibrous particulates. The collection efficiency of the sampler is not a function of flow rate in the range 0.5 to 16 L/min [10].
2. Sample Preparation Technique:
   The acetone vapor-triacetin preparation technique is a faster, more permanent mounting technique than the dimethyl phthalate/diethyl oxalate method of P&CAM 239 [2,4,10]. The aluminum "hot block" technique minimizes the amount of acetone needed to prepare each sample.
3. Measurement:
   a. The Walton-Beckett graticule standardizes the area observed [14,18,19].
   b. The HSE/NPL test slide standardizes microscope optics for sensitivity to fiber diameter [4,14].
   c. Because of past inaccuracies associated with low fiber counts, the minimum recommended loading has been increased to 100 fibers/mm^2 filter area (a total of 78.5 fibers counted in 100 fields, each with field area = .00785 mm^2.) Lower levels generally result in an overestimate of the fiber count when compared to results in the recommended analytical range [20]. The recommended loadings should yield intracounter S_r in the range of 0.10 to 0.17 [21,22,23].

B. Interlaboratory comparability:
An international collaborative study involved 16 laboratories using prepared slides from the asbestos cement, milling, mining, textile, and friction material industries [9]. The relative standard deviations (S_r) varied with sample type and laboratory. The ranges were:

	Intralaboratory S_r	Interlaboratory S_r	Overall S_r
AIA (NIOSH A Rules)*	0.12 to 0.40	0.27 to 0.85	0.46
Modified CRS (NIOSH B Rules)**	0.11 to 0.29	0.20 to 0.35	0.25

* Under AIA rules, only fibers having a diameter less than 3 µm are counted and fibers attached to particles larger than 3 µm are not counted. NIOSH A Rules are otherwise similar to the AIA rules.
** See Appendix C.

A NIOSH study conducted using field samples of asbestos gave intralaboratory S_r in the range 0.17 to 0.25 and an interlaboratory S_r of 0.45 [21]. This agrees well with other recent studies [9,14,16].

At this time, there is no independent means for assessing the overall accuracy of this method. One measure of reliability is to estimate how well the count for a single sample agrees with the mean count from a large number of laboratories. The following discussion indicates how this estimation can be carried out based on measurements of the interlaboratory variability, as well as showing how the results of this method relate to the theoretically attainable counting precision and to measured intra- and interlaboratory S_r. (NOTE: The following discussion does not include bias estimates and should not be taken to indicated that lightly loaded samples are as accurate as properly loaded ones).

Theoretically, the process of counting randomly (Poisson) distributed fibers on a filter surface will give an S_r that depends on the number, N, of fibers counted:

$$S_r = 1/(N)^{1/2} \qquad (1)$$

Thus S_r is 0.1 for 100 fibers and 0.32 for 10 fibers counted. The actual S_r found in a number of studies is greater than these theoretical numbers [17,19,20,21].

An additional component of variability comes primarily from subjective interlaboratory differences. In a study of ten counters in a continuing sample exchange program, Ogden [15] found this subjective component of intralaboratory S_r to be approximately 0.2 and estimated the overall S_r by the term:

$$\frac{[N + (0.2 \cdot N)^2]^{1/2}}{N} \qquad (2)$$

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

In a study of field sample results in 46 laboratories, the Asbestos Information Association also found that the variability had both a constant component and one that depended on the fiber count [14]. These results gave a subjective interlaboratory component of S_r (on the same basis as Ogden's) for field samples of ca. 0.45. A similar value was obtained for 12 laboratories analyzing a set of 24 field samples [21]. This value falls slightly above the range of S_r (0.25 to 0.42 for 1984-85) found for 80 reference laboratories in the NIOSH PAT program for laboratory-generated samples [17].

A number of factors influence S_r for a given laboratory, such as that laboratory's actual counting performance and the type of samples being analyzed. In the absence of other information, such as from an interlaboratory quality assurance program using field samples, the value for the subjective component of variability is chosen as 0.45. It is hoped that the laboratories will carry out the recommended interlaboratory quality assurance programs to improve their performance and thus reduce the S_r.

The above relative standard deviations apply when the population mean has been determined. It is more useful, however, for laboratories to estimate the 90% confidence interval on the mean count from a single sample fiber count (Figure 1). These curves assume similar shapes of the count distribution for interlaboratory and intralaboratory results [16].

For example, if a sample yields a count of 24 fibers, Figure 1 indicates that the mean interlaboratory count will fall within the range of 227% above and 52% below that value 90% of the time. We can apply these percentages directly to the air concentrations as well. If, for instance, this sample (24 fibers counted) represented a 500-L volume, then the measured concentration is 0.02 fibers/mL (assuming 100 fields counted, 25-mm filter, 0.00785 mm^2 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 fiber/mL. These limits should be reported in any comparison of results between laboratories.

Note that the S_r of 0.45 used to derive Figure 1 is used as an estimate for a random group of laboratories. If several laboratories belonging to a quality assurance group can show that their interlaboratory S_r is smaller, then it is more correct to use that smaller S_r. However, the estimated S_r of 0.45 is to be used in the absence of such information. Note also that it has been found that S_r can be higher for certain types of samples, such as asbestos cement [9].

Quite often the estimated airborne concentration from an asbestos analysis is used to compare to a regulatory standard. For instance, if one is trying to show compliance with an 0.5 fiber/mL standard using a single sample on which 100 fibers have been counted, then Figure 1 indicates that the 0.5 fiber/mL standard must be 213% higher than the measured air concentration. This indicates that if one measures a fiber concentration of 0.16 fiber/mL (100 fibers counted), then the mean fiber count by a group of laboratories (of which the compliance laboratory might be one) has a 95% chance of being less than 0.5 fibers/mL; i.e., 0.16 + 2.13 x 0.16 = 0.5.

It can be seen from Figure 1 that the Poisson component of the variability is not very important unless the number of fibers counted is small. Therefore, a further approximation is to simply use +213% and −49% as the upper and lower confidence values of the mean for a 100-fiber count.

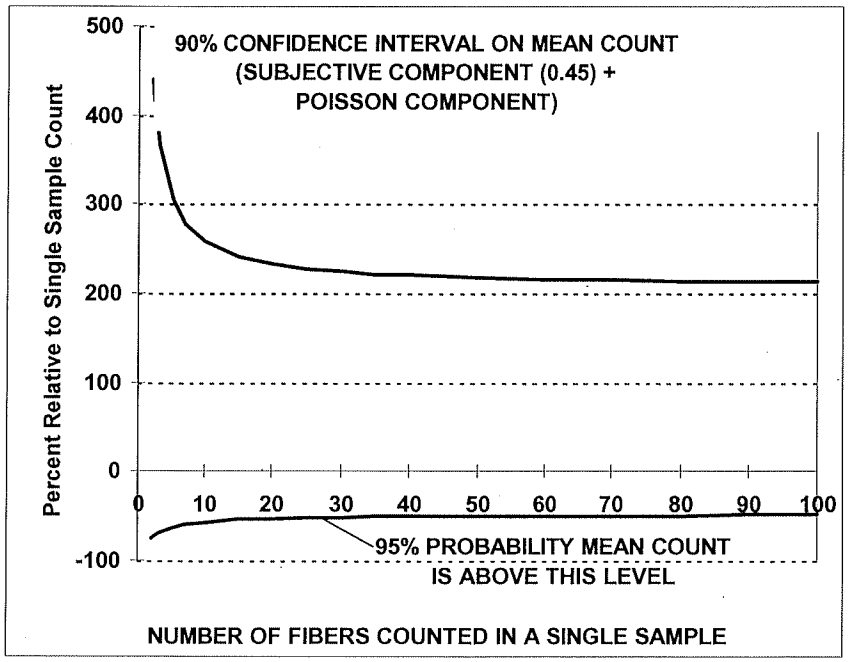

Figure 1. Interlaboratory Precision of Fiber Counts

The curves in Figures 1 are defined by the following equations:

$$UCL = \frac{2X + 2.25 + [(2.25 + 2X)^2 - 4(1 - 2.25 S_r^2) X^2]^{1/2}}{2(1 - 2.25 S_r^2)} \quad (3)$$

$$LCL = \frac{2X + 4 - [(4 + 2X)^2 - 4(1 - 4 S_r^2) X^2]^{1/2}}{2(1 - 4 S_r^2)} \quad (4)$$

where S_r = subjective interlaboratory relative standard deviation, which is close to the total interlaboratory S_r when approximately 100 fibers are counted.
X = total fibers counted on sample
LCL = lower 95% confidence limit.
UCL = upper 95% confidence limit.
Note that the range between these two limits represents 90% of the total range.

REFERENCES:

[1] Leidel, N. A., S. G. Bayer, R. D. Zumwalde, and K. A. Busch. USPHS/NIOSH Membrane Filter Method for Evaluating Airborne Asbestos Fibers, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 79-127 (1979).

[2] Baron, P. A. and G. C. Pickford. "An Asbestos Sample Filter Clearing Procedure," Appl. Ind. Hyg., 1:169-171, 199 (1986).

[3] Occupational Safety and Health Administration, U.S. Department of Labor, Occupational Exposure to Asbestos, Tremolite, Anthophyllite, and Actinolite Asbestos; Final Rules, 29 CFR Part 1910.1001 Amended June 20, 1986.

[4] Rooker, S. J., N. P. Vaughn, and J. M. LeGuen. "On the Visibility of Fibers by Phase Contrast Microscopy," Amer. Ind. Hyg. Assoc. J., 43, 505-515 (1982).

[5] Baron, P. and G. Deye, "Electrostatic Effects in Asbestos Sampling," Parts I and II Amer. Ind. Hyg. Assoc. J., 51, 51-69 (1990).

[6] Johnston, A. M., A. D. Jones, and J. H. Vincent. "The Influence of External Aerodynamic Factors on the Measurement of the Airborne Concentration of Asbestos Fibers by the Membrane Filter Method," Ann. Occup. Hyg., 25, 309-316 (1982).

[7] Beckett, S.T., "The Effects of Sampling Practice on the Measured Concentration of Airborne Asbestos," Ann. Occup. Hyg., 21, 259-272 (1980).

[8] Jankovic, J. T., W. Jones, and J. Clere. "Field Techniques for Clearing Cellulose Ester Filters Used in Asbestos Sampling," Appl. Ind. Hyg., 1, 145-147 (1986).

[9] Crawford, N. P., H. L. Thorpe, and W. Alexander. "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. TM/82/23), Part II: Effects on Reproducibility (Report No. TM/82/24), Institute of Occupational Medicine, Edinburgh, Scotland (December, 1982).

[10] NIOSH Manual of Analytical Methods, 2nd ed., Vol. 1., P&CAM 239, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-A (1977).

[11] Revised Recommended Asbestos Standard, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-169 (1976); as amended in NIOSH statement at OSHA Public Hearing, June 21, 1984.

[12] Asbestos International Association, AIA Health and Safety Recommended Technical Method #1 (RTMI). "Airborne Asbestos Fiber Concentrations at Workplaces by Light Microscopy" (Membrane Filter Method), London (1979).

[13] Abell, M., S. Shulman and P. Baron. The Quality of Fiber Count Data, Appl. Ind. Hyg., 4, 273-285

(1989).
[14] "A Study of the Empirical Precision of Airborne Asbestos Concentration Measurements in the Workplace by the Membrane Filter Method," Asbestos Information Association, Air Monitoring Committee Report, Arlington, VA (June, 1983).
[15] McCrone, W., L. McCrone and J. Delly, "Polarized Light Microscopy," Ann Arbor Science (1978).
[16] Ogden, T. L. "The Reproducibility of Fiber Counts," Health and Safety Executive Research Paper 18 (1982).
[17] Schlecht, P. C. and S. A. Schulman. "Performance of Asbestos Fiber Counting Laboratories in the NIOSH Proficiency Analytical Testing (PAT) Program," Am. Ind. Hyg. Assoc. J., 47, 259-266 (1986).
[18] Chatfield, E. J. Measurement of Asbestos Fiber Concentrations in Workplace Atmospheres, Royal Commission on Matters of Health and Safety Arising from the Use of Asbestos in Ontario, Study No. 9, 180 Dundas Street West, 22nd Floor, Toronto, Ontario, CANADA M5G 1Z8.
[19] Walton, W. H. "The Nature, Hazards, and Assessment of Occupational Exposure to Airborne Asbestos Dust: A Review," Ann. Occup. Hyg., 25, 115-247 (1982).
[20] Cherrie, J., A.D. Jones, and A.M. Johnston. "The Influence of Fiber Density on the Assessment of Fiber Concentration Using the membrane filter Method." Am. Ind. Hyg. Assoc. J., 47(8), 465-74 (1986).
[21] Baron, P. A. and S. Shulman. "Evaluation of the Magiscan Image Analyzer for Asbestos Fiber Counting." Am. Ind. Hyg. Assoc. J., (in press).
[22] Taylor, D. G., P. A. Baron, S. A. Shulman and J. W. Carter. "Identification and Counting of Asbestos Fibers," Am. Ind. Hyg. Assoc. J. 45(2), 84-88 (1984).
[23] "Potential Health Hazards of Video Display Terminals," NIOSH Research Report, June 1981.
[24] "Reference Methods for Measuring Airborne Man-Made Mineral Fibers (MMMF)," WHO/EURO Technical Committee for Monitoring an Evaluating Airborne MMMF, World Health Organization, Copenhagen (1985).
[25] Criteria for a Recommended Standard...Occupational Exposure to Fibrous Glass, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-152 (1977).

METHOD WRITTEN BY:

Paul A. Baron, Ph.D., NIOSH/DPSE.

APPENDIX A: CALIBRATION OF THE WALTON-BECKETT GRATICULE:

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

1. Insert any available graticule into 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 so that the magnification remains constant.
3. Install the 40 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 magnified grid length of the graticule, L_o (µm), using the stage micrometer.
6. Remove the graticule from the microscope and measure its actual grid length, L_a (mm). This can best be accomplished by using a stage fitted with verniers.
7. Calculate the circle diameter, d_c (mm), for the Walton-Beckett graticule:

$$d_c = \frac{L_a}{L_o} \times D. \qquad (5)$$

Example: If L_o = 112 µm, L_a = 4.5 mm and D = 100 µm, then d_c = 4.02 mm.

8. Check the field diameter, D (acceptable range 100 µm ± 2 µm) with a stage micrometer upon receipt of the graticule from the manufacturer. Determine field area (acceptable range 0.00754 mm^2 to 0.00817 mm^2).

APPENDIX B: COMPARISON OF COUNTING RULES:

Figure 2 shows a Walton-Beckett graticule as seen through the microscope. The rules will be discussed as they apply to the labeled objects in the figure.

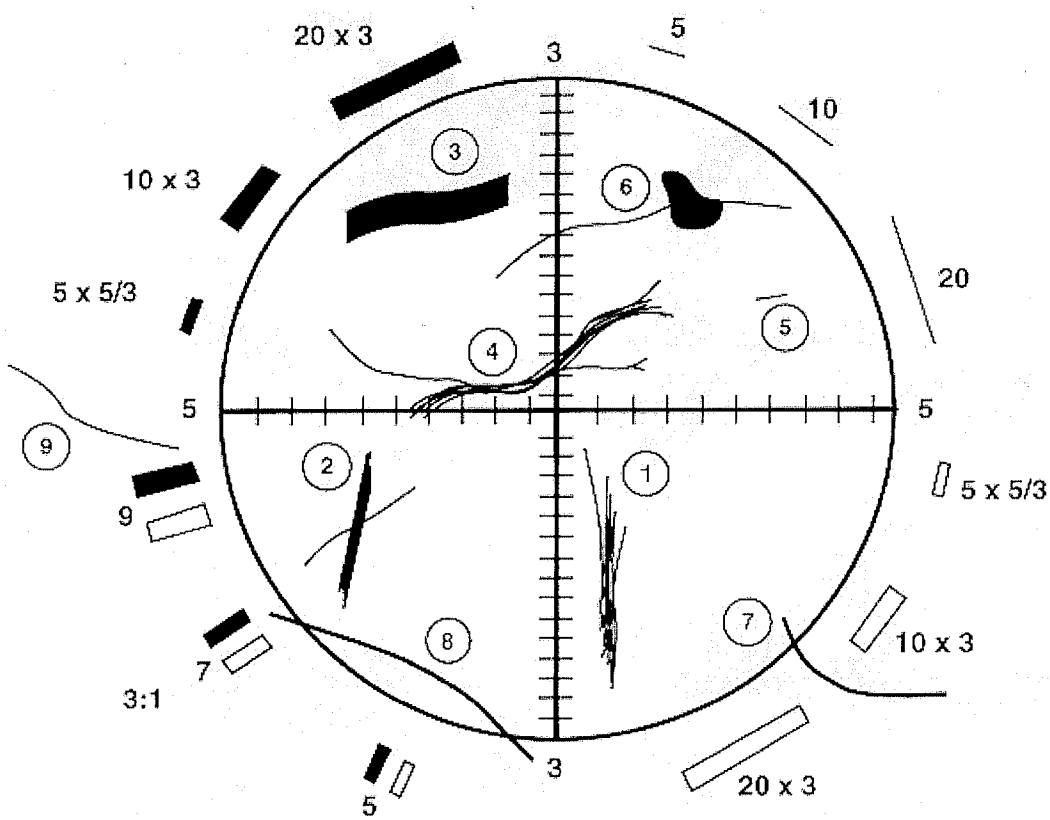

Figure 2. Walton-Beckett graticule with fibers.

These rules are sometimes referred to as the "A" rules.

FIBER COUNT

Object	Count	DISCUSSION
1	1 fiber	Optically observable asbestos fibers are actually bundles of fine fibrils. If the fibrils seem to be from the same bundle the object is counted as a single fiber. Note, however, that all objects meeting length and aspect ratio criteria are counted whether or not they appear to be asbestos.
2	2 fiber	If fibers meeting the length and aspect ratio criteria (length >5 µm and length-to-width ratio >3 to 1) overlap, but do not seem to be part of the same bundle, they are counted as separate fibers.
3	1 fiber	Although the object has a relatively large diameter (>3 µm), it is counted as fiber under the rules. There is no upper limit on the fiber diameter in the counting rules. Note that fiber width is measured at the widest compact section of the object.
4	1 fiber	Although long fine fibrils may extend from the body of a fiber, these fibrils are considered part of the fiber if they seem to have originally been part of the bundle.
5	Do not count	If the object is ≤5 µm long, it is not counted.
6	1 fiber	A fiber partially obscured by a particle is counted as one fiber. If the fiber ends emanating from a particle do not seem to be from the same fiber and each end meets the length and aspect ratio criteria, they are counted as separate fibers.
7	1/2 fiber	A fiber which crosses into the graticule area one time is counted as 1/2 fiber.
8	Do not count	Ignore fibers that cross the graticulate boundary more than once. count
9	Do not count	Ignore fibers that lie outside the graticule boundary.

APPENDIX C. ALTERNATE COUNTING RULES FOR NON-ASBESTOS FIBERS

Other counting rules may be more appropriate for measurement of specific non-asbestos fiber types, such as fibrous glass. These include the "B" rules given below (from NIOSH Method 7400, Revision #2, dated 8/15/87), the World Health Organization reference method for man-made mineral fiber [24], and the NIOSH fibrous glass criteria document method [25]. The upper diameter limit in these methods prevents measurements of non-thoracic fibers. It is important to note that the aspect ratio limits included in these methods vary. NIOSH recommends the use of the 3:1 aspect ratio in counting fibers.

It is emphasized that hybridization of different sets of counting rules is not permitted. Report specifically which set of counting rules are used with the analytical results.

"B" COUNTING RULES:

1. Count only ends of fibers. Each fiber must be longer than 5 µm and less than 3 µm diameter.
2. Count only ends of fibers with a length-to-width ratio equal to or greater than 5:1.
3. Count each fiber end which falls within the graticule area as one end, provided that the fiber meets rules 1 and 2 above. Add split ends to the count as appropriate if the split fiber segment also meets the criteria of rules 1 and 2 above.
4. Count visibly free ends which meet rules 1 and 2 above when the fiber appears to be attached to another particle, regardless of the size of the other particle. Count the end of a fiber obscured by another particle if the particle covering the fiber end is less than 3 µm in diameter.
5. Count free ends of fibers emanating from large clumps and bundles up to a maximum of 10 ends (5 fibers), provided that each segment meets rules 1 and 2 above.
6. Count enough graticule fields to yield 200 ends. Count a minimum of 20 graticule fields. Stop at 100 graticule fields, regardless of count.
7. Divide total end count by 2 to yield fiber count.

APPENDIX D. EQUIVALENT LIMITS OF DETECTION AND QUANTITATION

	fiber density on filter*		fiber concentration in air, f/cc	
	fibers per 100 fields	fibers/mm^2	400-L air sample	1000-L air sample
	200	255	0.25	0.10
	100	127	0.125	0.05
LOQ	80	102	0.10	0.04
	50	64	0.0625	0.025
	25	32	0.03	0.0125
	20	25	0.025	0.010
	10	12.7	0.0125	0.005
	8	10.2	0.010	0.004
LOD	5.5	7	0.00675	0.0027

* Assumes 385 mm^2 effective filter collection area, and field area = 0.00785 mm^2, for relatively "clean" (little particulate aside from fibers) filters.