fld Sampling Methods

NIOSH MANUAL OF ANALYTICAL METHODS

FORMULA: various

M.W.: various

METHOD: 7400
ISSUED: 2/15/84
REVISION #2: 3/1/87

OSHA: 0.2 asbestos fibers (> 5 μm long)/mL
NIOSH: 0.1 asbestos f/mL [1]; 3 glass fibers (>10 μm x <3.5 μm)/mL [2]
ACGIH: 0.2 crocidolite; 0.5 amosite; 2 chrysotile and other asbestos, f/mL

PROPERTIES: solid, fibrous

SYNONYMS: actinolite asbestos [CAS #13768-00-8], grunerite asbestos (amosite) [CAS #12172-73-5], anthophyllite asbestos [CAS #17068-78-9], chrysotile asbestos [CAS #12001-29-5], crocidolite asbestos [CAS #12001-28-4], tremolite asbestos [CAS #14567-73-8]; fibrous glass.

SAMPLING	MEASUREMENT
SAMPLER: FILTER (0.8 to 1.2 μm cellulose ester membrane, 25-mm diameter; conductive coal on cassette)	TECHNIQUE: LIGHT MICROSCOPY, POSITIVE PHASE CONTRAST
FLOW RATE*: 0.5 to 16 L/min (see step 4)	ANALYTE: fibers (manual count)
VOL-MIN*: 400 L @ 0.1 fiber/mL (see step 4) -MAX*: (see step 4) *Adjust for 100 to 1300 fibers/mm² (step 4)	SAMPLE PREPARATION: acetone/triacetin "hot block" method [4]
SHIPMENT: routine (securely packed to reduce shock)	COUNTING RULES: Set A (P&CAM 239 [3,4]) or Set B (modified CRS [5])
SAMPLE STABILITY: stable	EQUIPMENT: 1. positive phase-contrast microscope 2. Walton-Beckett graticule (100 μm field diameter): A Rules use Type G-22; B Rules use Type G-24 3. phase-shift test slide (HSE/NPL)
FIELD BLANKS: 10% (≥2) of samples	CALIBRATION: HSE/NPL test slide
ACCURACY	RANGE: 100 to 1300 fibers/mm² filter area
RANGE STUDIED: 80 to 100 fibers counted	ESTIMATED LOD: 7 fibers/mm² filter area
BIAS: see EVALUATION OF METHOD	PRECISION: 0.10 to 0.12 (A Rules) [3] (see Appendix C)
OVERALL PRECISION (s_r): 0.115 to 0.13 (A Rules) [3] (see Appendix C)	

APPLICABILITY: The method gives an index of airborne fibers in workplace atmospheres. Phase contrast microscopy will not differentiate between asbestos and other fibers; use this method in conjunction with electron microscopy (e.g., Method 7402) for positive identification. Fibers < ca. 0.25 μm diameter will not be detected by this method [6].

INTERFERENCES: 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 method introduces changes for improved sensitivity and reproducibility. It also replaces P&CAM 239 [3,7] and Method 7400 (dated 5/15/85).

REAGENTS:

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

*See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: field monitor, 25-mm, three-piece cassette with 50-mm extension coul with cellulose ester filter, 0.8- to 1.2-µm pore size and backup pad.
   NOTE 1: Analyze representative filters for fiber background before use and discard the filter lot if a mean of more than 5 fibers/100 fields are found. These are defined as laboratory blanks.
   NOTE 2: An electrically conductive extension coul is required to reduce electrostatic effects on fiber sampling. Ground the coul whenever possible during sampling.
2. Personal sampling pump, 0.5 to 16 L/min (see step 4 for flow rate), with flexible connecting tubing.
3. Microscope, positive phase contrast, with green or blue filter, 8 to 10X eyepiece, and 40 to 45X phase objective (total magnification ca. 400X); numerical aperture = 0.65 to 0.75.
4. Slides, glass, frosted-end, pre-cleaned, 25 x 75 mm.
5. Cover slips, 22 x 22 mm, No. 1-1/2, unless otherwise specified by microscope manufacturer.
6. Lacquer or nail polish.
7. Knife, #10 surgical steel, curved blade.
8. Tweezers.
9. Heated aluminum block for clearing filters on glass slides (see ref. [4] for instructions on manufacture).
10. Micropipet, 100- to 500-µL.
11. Micropipets, 5-µL.
12. Graticule, Walton-Beckett type with 100-µm diameter circular field at the specimen plane (area = 0.00785 mm^2) (Type G-22 for A Rules; Type G-24 for B Rules). Available from PTR Optics Ltd., 145 Newton Street, Waltham, MA 02154 (Telephone [617] 891-6000) and McCrone Accessories and Components, 2506 S. Michigan Ave., Chicago, IL 60616 (Telephone [312] 842-7100).
    NOTE: The graticule is custom-made for each microscope. Specify disc diameter needed to fit exactly the ocular of the microscope and the diameter (mm) of the circular counting area (see APPENDIX A).
13. HSE/NPL phase contrast test slide, Mark II. Available from PTR Optics Ltd.
14. Telescope, ocular phase-ring centering.
15. Stage micrometer (0.01-mm divisions).
16. Wire, multi-stranded, 22-gauge.

SPECIAL PRECAUTIONS: Acetone is extremely flammable liquid. Take precautions must be taken 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 [3].
2. For personal sampling, fasten the sampler to the worker's lapel as close as possible to the worker's mouth. Remove the top cover from the end of the cowl extension (open face) and orient face down. Wrap the joint between the cowl and monitor body with shrink tape to prevent air leaks.
   NOTE: Use a conductive cowl and, if possible, ground the cassette to remove any surface charge. Grounding should consist of a wire held in contact (e.g., with a hose clamp) with the conductive cowl and attached to a non-electrical metal fixture.
3. Submit at least two field blanks (or 10% of the total samples, whichever is greater) for each set of samples. Remove the top covers from the field blank cassettes and store the top covers and cassettes in a clean area (bag or box) with the caps from the sampling cassettes during the sampling period. Replace the caps in the cassettes when sampling is completed.
4. Sample at 0.5 L/min or greater [8]. Adjust sampling flow rate, Q (L/min), and time, t (min), to produce a fiber density, E (fibers/mm^2), of 100 to 1300 fibers/mm^2 [3.85•10^4 to 5•10^5 fibers per 25-mm filter with effective collection area (A_c= 385 mm^2)] for optimum counting precision and quantitation (step 20). If more than 50% of the filter surface is covered wth particles, the filter may be too overloaded to count.. These variables are related to the action level (one-half the current standard), L (fibers/mL), of the fibrous aerosol being sampled by:

$$t = \frac{(A_c)(E)}{(Q)(L)10^3}$$

   NOTE: A sampling flow rate of 1 to 4 L/min for 8 hrs is appropriate in non-dusty atmospheres containing ca. 0.1 fiber/mL. Dusty atmospheres require smaller sample volumes; ambient, clean atmospheres require larger air samples for optimum counting precision. For documenting episodic exposures, higher flow rates may be used over shorter collection times. The primary consideration in calculating sampling times is to obtain optimum loading on the filter. The sample volumes needed to achieve optimum loadings will vary considerably between atmospheres of high and low fiber concentrations. It is conceivable that in dusty areas, sample volumes of less than 400 L may be needed to obtain countable samples. In such cases it may be necessary to take short, consecutive samples and average the results over the total collection time. For documenting episodic exposures, high flow rates (around 7-16 L/min) may be needed over shorter sampling times. Also, in relatively clean atmospheres, where targeted fiber concentrations are much less than 0.1 fibes/mL, much larger sample volumes (on the order of 3000 to 10000 L) may be needed to achieve quantifiable fiber loadings on the filters. Care should be taken, however, not to overload the filter with background dust.
5. Remove the field monitor at the end of sampling, replace the plastic top cover and small end caps, and store the monitor.
6. Ship the samples in a rigid container with sufficient packing material to prevent jostling or damage.
   NOTE: Do not use untreated polystyrene foam in the shipping container because electrostatic forces may cause fiber loss from the sampler filter.

SAMPLE PREPARATION:

Mounting Procedure:

NOTE: The object is to produce samples with a smooth (non-grainy) background in a medium with a refractive index equal to or less than 1.46. The method below collapses the filter for easier focusing and produces permanent mounts which are useful for quality control and interlaboratory comparison. The aluminum "hot block" technique is a method for mounting cellulose ester filters outside the laboratory. 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 [1,3,7,21]). A videotape of the mounting procedure is available from the NIOSH Publication Office [19].

7. Ensure that the glass slides and cover slips are free of dust and fibers.
8. Plug in and turn on the heat source to the aluminum "hot block" clearing device [4]. Adjust the rheostat to heat the block to approximately 70 °C. If the "hot block" is not used in a fume hood, it must rest on a ceramic plate and be isolated from any surface which is 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 steel surgical knife using a rocking motion to prevent tearing.
   b. Place the filter or wedge, dust side up, on the slide. Static electricity will usually keep the filter on the slide until it is cleared.
   c. Insert the glass slide into the receiving slot at the base of the heated aluminum block. Place the tip of a micropipet containing ca. 250 µL acetone into the inlet port of the PTFE cap on top of the aluminum block. Inject the acetone into the vaporization chamber with a slow, steady pressure on the plunger button while holding the pipet firmly in place. Remove the pipet and glass slide from their respective ports after waiting 3 to 5 sec for the filter to clear.
      CAUTION: Although the volume of acetone used in this technique is small, it is, nevertheless, incumbent on the user to take the necessary safety precautions to ensure safe use of the mounting technique. It is recommended that the block be used inside a laboratory fume hood when available. If the "hot block" device is used outside of a fume hood, take precautions not to ignite the acetone and to keep the mounting area well ventilated. Continuous frequent use of this device in a closed or unventilated space may produce acetone vapor build-up and is not recommended.
   d. Using the 5-µL micropipet, immediately place 3.0 to 3.5 µL triacetin on the filter. Gently lower a clean cover slip down onto the filter at a slight angle to reduce the possibility of forming bubbles.
      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 in contact with the edge of the filter under the cover slip, fiber migration may occur at the edges.
   e. Glue the edges of the cover slip to the glass slide using a lacquer or nail polish [9].
      NOTE: If clearing is slow, the slide may be warmed on a hotplate (surface temperature 50 °C) for up to 15 min to hasten clearing. Use caution when heating a prepared filter to ensure that gas bubbles do not form in the sample preparation. Counting may proceed immediately after clearing and mounting are completed.

CALIBRATION AND QUALITY CONTROL:

10. Microscope adjustments. With each microscope, keep a logbook which records the dates that microscope cleanings, adjustments, and calibrations are made. Follow the manufacturer's instructions and, each time a sample is examined, do the following:
    a. Adjust the light source for even illumination across the field of view at the condenser iris each time a new sample is examined.
       NOTE: Köhler illumination is preferred, where available.
    b. Focus on the particulate material to be examined.
    c. Make sure that the field iris is in focus, centered on the sample, and open only enough to fully illuminate the field of view.
    d. Use the telescope ocular supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric. Perform this check at least once daily.

11. Check the phase-shift detection limit of the microscope periodically for each analyst/microscope combination:
    a. Remove the HSE/NPL phase-contrast test slide from its shipping container and center it under the phase objective.
    b. Bring the blocks of grooved lines into focus.
       NOTE: The slide consists of seven blocks of grooves (ca. 20 grooves to each block) in descending order of visibility from sets 1 to 7. The requirements for asbestos counting are that the microscope optics must resolve the grooved lines in set 3 completely, although they may appear somewhat faint, and that the grooved lines in sets 6 and 7 must be invisible. Sets 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 either too low or too high a resolution to be used for fiber counting.
    c. If the image quality deteriorates, clean the microscope optics. If the problem persists, consult the microscope manufacturer.

12. Quality control of fiber counts.
    a. Prepare and count field blanks along with the field samples. Report the counts on each blank. Calculate the mean of the field blank counts and subtract this value from each sample count before reporting the results.
       NOTE 1: The identity of the blank filters should be unknown to the counter until all counts have been completed.
       NOTE 2: If a field blank yields fiber counts greater than 7 fibers/100 fields, report possible contamination of the samples.
    b. Perform blind recounts by the same counter on 10% of filters counted (slides relabeled by a person other than the counter).
    c. Each laboratory should maintain as an integral part of its quality assurance program a set of reference slides to be used on a daily basis. These slides should consist of filter preparations from a variety of sources including both field and PAT samples. The set of reference slides should be large enough to accomodate a range of loadings and background dust levels typically encountered on a routine basis by the laboratory. The Quality Assurance Officer should maintain custody of the reference slides and ensure that each counter is supplied with a minimum of one reference slide per workday as a quality control procedure. The labels on the refence slides should be changed periodically so that the analyst does not become familiar with the samples. By obtaining records of blind repeat counts on these reference slides, a laboratory can document its internal intra- and inter-counter s_r (see step 21).

13. 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. This statistic estimates the counting repeatability at the 95% confidence level. Discard the sample if the difference between the two counts exceeds 2.77 $(\bar{F})s_r$, where $\bar{F}$ = average of the two fiber counts and s_r = relative standard deviation, which should be derived by each laboratory based on historical in-house data.
    NOTE: If a pair of counts is rejected as a result of 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. Enroll each new counter in a training course which compares performance of counters on a variety of samples using this procedure.
    NOTE: To ensure good reproducibility, all laboratories engaged in asbestos counting should participate in an asbestos proficiency testing program such as the NIOSH Proficiency Analytical Testing (PAT) Program and routinely participate with other asbestos fiber counting laboratories in the exchange of field samples to compare performance of counters.

MEASUREMENT:

15. Place the slide on the mechanical stage of the calibrated microscope with the center of the filter under the objective lens. Focus the microscope on the plane of the filter.
16. Regularly check and optimize phase-ring alignment and Köhler illumination [6].
17. Select one of the following sets of counting rules:
    NOTE: The two sets of rules have been demonstrated to produce equivalent mean counts on a variety of asbestos sample types [5] and must be strictly followed in order to obtain valid results. No hybridizing of the two sets of rules is permitted. The calibration of the microscope with the HSE/NPL test slide determines the minimum detectable fiber diameter (ca. 0.25 µm).
    a. A Rules (same as P&CAM 239 rules [1,3,7]; see APPENDIX B).
        1. Count only fibers longer than 5 µm. Measure the length of curved fibers along the curve.
        2. Count only fibers with a length-to-width ratio equal to or greater than 3:1.
        3. For fibers which cross the boundary of the graticule field, do the following:
            a. Count any fiber longer than 5 µm which lies entirely within the graticule area.
            b. Count as 1/2 fiber any fiber with only one end lying within the graticule area, if the fiber meets the criteria of rules a.1. and a.2.
            c. Do not count any fiber which crosses the graticule boundary more than once.
            d. Reject and do not count all other fibers.
        4. Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of a fiber.
        5. Count enough graticule fields to yield 100 fibers. Count a minimum of 20 fields. Stop at 100 fields regardless of fiber count.
    b. B Rules (see APPENDIX B)
        NOTE: The B Rules are preferred analytically because of their demonstrated ability to improve the reproducibility of fiber counts [5].
        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 b.1 and b.2. Add split ends to the count as appropriate if the split fiber segment also meets the dimensional criteria in rules b.1 and b.2.

4. Count visibly free ends which meet rules b.1 and b.2 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 the 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 b.1 and b.2.
6. Count enough graticule fields to yield 200 ends. Count a minimum of 20 fields. Stop at 100 fields, regardless of the end count.
7. Divide the total end count by 2 to yield fiber count.

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

NOTE 1: When counting a 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/field is appropriate to ensure thorough sample examination.

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 acceptable method for ensuring uniformity of judgement between laboratories. It is, therefore, incumbent upon all laboratories using this method to report <u>total</u> fiber counts. Other techniques such as Polarized Light Microscopy are available which can identify and eliminate some fiberglass and other isotropic fibers having diameters larger than ca. 2 μm from the fiber count. If such procedures are used, they should be noted in the report of analytical results. If serious contamination from non-asbestos fibers occurs in samples used to obtain asbestos fiber counts, other techniques such as Transmission Electron Microscopy must be used to identify the asbestos fiber fraction present in the sample (see NIOSH Method 7402).

CALCULATIONS AND REPORTING OF DATA:

19. Calculate and report fiber density on the filter, E (fibers/mm^2), by dividing the total fiber count, F, minus the mean field blank count, B, by the number of fields, n, and the field area, A_f (0.00785 mm^2 for a properly calibrated Walton-Beckett graticule):

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

20. Calculate the concentration, C (fibers/mL), of fibers in the air volume sampled, V (L), using the effective collection area of the filter, A_c (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.

21. Report intralaboratory relative standard deviation (s_r) with each set of results. Because precision is dependent upon the total number of fibers counted [3,10], it is insufficient to report only a number for fiber air concentration when using either Method 7400 or P&CAM 239. So that the end user can best utilize the data, laboratories are obligated to report both fiber concentrations as a function of filter area and air volume, and the reliability of the reported numbers in terms of precision. Relative standard deviation (also called coefficient of variation) is documented in references [3,10,11,12] for fiber counts up to 100 fibers in 100 fields. In addition, each laboratory should maintain records which document the laboratory's s_r for fiber counts in the quantitative range of 100 to 1300 fibers/mm^2. This will facilitate control of data within the laboratory, help the Quality Assurance Officer to identify problems and be useful to the end user of the data. In order to estimate comparability of interlaboratory results, see APPENDIX C. 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. 2).

EVALUATION OF METHOD:

This method is a revision of NIOSH Method P&CAM 239 [1,3,7]. A summary of the revisions is as follows:

A. Sampling:
The change from a 37-mm to a 25-mm filter size was incorporated to improve 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 affected by changes in flow rate in the range 0.5 to 16 L/min [8].

B. Sample Preparation Technique:
The acetone vapor-triacetin preparation technique has been incorporated in the method as a faster, more permanent mounting technique than the dimethyl phthalate/diethyl oxalate method of P&CAM 239 [1,3,4,7,13]. The aluminum "hot block" technique minimizes the amount of acetone needed to prepare each sample.

C. Measurement:
1. The inclusion of the Walton-Beckett graticule in the method was made to standardize the field area observed through the eyepiece [13,14].
2. The introduction of the HSE/NPL test slide was made to standardize microscope optics for sensitivity to fiber diameter [6,13].
3. An international collaborative study involved 16 laboratories using prepared slides from the asbestos, cement, milling, mining, textile, and friction material industries [5]. The modified CRS (NIOSH B) Rules were found to yield equivalent counts but were more precise than the AIA (NIOSH A)* Rules. The relative standard deviations (s_r) varied with sample type and laboratory. The ranges were:

	s_r		
	Intralaboratory	Interlaboratory	Overall
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.

4. The B Rules have also been favorably received by analysts as less ambiguous and simpler to use; these rules also showed the least bias relative to AIA rules in the collaborative study. An independent NIOSH laboratory study using amosite fibers reported a relative standard deviation, including within- and between-sample variability, of 0.16 for the B Rules [15]. Another NIOSH study was conducted using field samples of asbestos [18]. This study indicated intralaboratory s_r's in the range 0.17-0.25 and an interlaboratory s_r of 0.45. This data agrees well with results reported from other recent studies [5,10,12] (see Appendix C).
5. Because of past inaccuracies associated with low fiber counts, the minimum recommended loading has been increased to 100 fibers/mm^2 filter area (80 fibers total count). This level should yield intra-counter s_r in the range of 0.13 to 0.17 [3,7,15,18]. APPENDIX C discusses interlaboratory accuracy and precision.

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APPENDIX A:

CALIBRATION OF THE WALTON-BECKETT GRATICULE:

Calibration of the Walton-Beckett graticule 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:

$$d_c = \frac{L_a}{L_o} \times D.$$

Example: If L_o = 108 µm, L_a = 2.93 mm and D = 100 µm, then d_c = 2.71 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 (mm²).

APPENDIX B:

COMPARISON OF COUNTING RULES:

Figure 1 represents a Walton-Beckett graticule as seen through the eyepiece of the microscope. Although the graticule design shown accomodates counting rules using the 3:1 aspect ratio definition for a fiber, both the "A" and "B" rules will be discussed as they apply to the labeled fibers in the figure.

FIBER COUNT

Fiber	A Rules	B Rules	DISCUSSION
1	1 fiber	3 ends	(A) No accomodation is made in the "A" rules for split ends; therefore, count one fiber. (B) When using the 'B' rules, first detemine whether the fiber meets the necessary dimensional criteria, i.e., >5 µm, >5:1 aspect ratio, <3 µm diameter. Next determine which two ends are the main trunk of the fiber and count as two ends. Finally, count all split ends which are greater than 5 µm as one end. Fiber #1 in this figure is counted as 3 ends under the "B" rules.
2	1 fiber	2 ends	(A) Single fiber with a small particle attached. The particle is not considered by the "A" rules and treated as if it does not exist. (B) The particle is less than 3 µm in diameter and, therefore, ignored under the "B" rules.
3	1 fiber	2 ends	(A) As with Fiber 1, one fiber is counted under "A" rules because it meets the >3:1 aspect ratio, >5 µm criteria. (B) The split end is less than 5 µm long so it is not counted under the "B" rules.
4	1 fiber	5 ends	(A) Fiber ends all are attached to a central large fiber or bundle; therefore, one fiber is counted under "A" rules. (B) Two ends are counted as belonging to the main fiber. Three of the remaining four split ends are >5 µm, giving a total of 5 ends under the "B" rules.
5	1 fiber	Do not count	(A) No diameter limit under "A" rules; therefore this thick fiber is counted because it meets the >3:1, >5 µm counting criteria. (B) The fiber is >3 µm diameter; therefore, it is not counted under the "B" rules.
6	1 fiber	1 end	(A) Non-fibrous particulate matter is treated as non-existent under the "A" rules; therefore, this fiber is counted as a whole fiber. (B) The short end of the fiber is less <5 µm long and obscured by a particle >3 µm in diameter; therefore, not counted under the "B" rules.

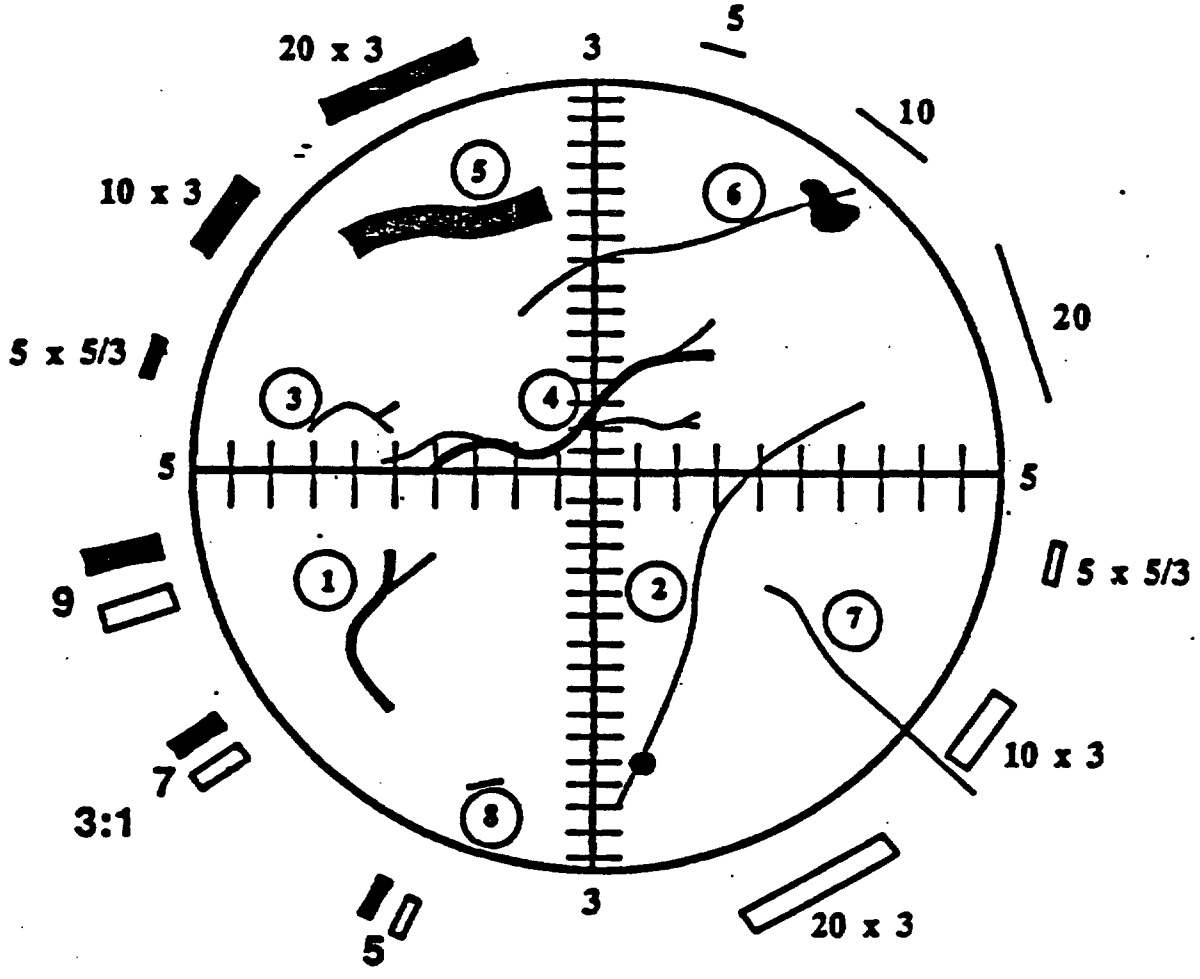

Figure 1. Walton-Beckett graticule with fibers.

Fiber	A Rules	B Rules	DISCUSSION
7	1/2 fiber	1 end	(A) Fibers which meet the definition of a fiber in rules a.1. and a.2. and cross the graticule boundary are counted as 1/2 fiber under the "A" rules unless the fiber crosses the graticule boundary more than once, in which case the fiber is not counted no matter how many ends lie within the graticule area. (B) Fiber ends lying inside the graticule boundary are counted as one end provided that the entire fiber meets the definition of a fiber in rules b.1. and b.2. and each end is longer than 5 µm. The portion of the fiber lying outside the graticule boundary must be considered in order to make this determination. Under "B" rules, it does not matter how often the fiber crosses the graticule boundary.
8	Do not count	Do not count	The fiber is <5 µm long.

APPENDIX C:

INTERLABORATORY COMPARABILITY:

At this time, there is no independent analytical method for analyzing asbestos so that the overall accuracy of the current method can be determined. Therefore, it is desirable to have some means of estimating the reliability of results determined by this method. One way of assessing this 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 inter-laboratory variability, as well as showing how the results of this technique relate to the theoretically attainable counting precision and to measured intra- and inter-laboratory s_r.

Theoretically, the process of counting randomly (Poisson) distributed fibers on a filter surface will give a coefficient of variation (s_r) that depends on the number (N) of fibers counted

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

This gives s_r of 0.1 for 100 fibers and 0.32 for 10 fibers counted. The actual fiber count precision (s_r) found in a number of studies is greater than these theoretical numbers (5,10,11,12).

There is an additional component of variability that comes primarily from "subjective" differences from counter to counter, and, more importantly, from laboratory to laboratory. In a study of a group of ten counters that were part of a continuing sample exchange program, Ogden (10) found this subjective component of intra-laboratory 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 intra-laboratory 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 assurance sample. For laboratories not engaged in an intensive quality assurance program, the subjective component of variability can be higher.

In a study of counting field samples by 46 laboratories, the Asbestos Information Association (12) also found that the variability had both a constant component and one that depended on the fiber count. These results indicate the subjective s_r (on the same basis as the Ogden analysis) for field samples of approximately 0.45 (subjective component of inter-laboratory s_r). This is the same s_r obtained for 12 PAT laboratories analyzing a set of 24 field samples (18). This value falls slightly above the range of s_r's (0.25 - 0.42 for 1984-85) found for 80 reference laboratories in the NIOSH Proficiency Analytical Testing program (laboratory generated samples) (11).

Obviously, a number of factors come into play in the determination of s_r for a reporting 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 inter-laboratory quality assurance program using field samples, the value for the subjective component of variability is chosen as 0.45. Note that, though based on at least two studies, this is a somewhat arbitrary choice. It is hoped that by requiring the use of this number in the absence of other information, laboratories will carry out the recommended inter-laboratory quality assurance programs to improve their performance and thus reduce the s_r that they use.

The above s_r's describe the range of counts when the population mean has been determined. It is more useful for laboratories reporting results to estimate the 90% confidence interval on the mean count from a single sample fiber count. Given a single fiber count, Figure 2 shows the range in which it is estimated that 90% of the inter-laboratory means will fall. These curves were calculated assuming that the shape of the count distribution for inter-laboratory results is the same as that for intra-laboratory results. For further details on how these curves were calculated see Ogden (10).

For example, if a sample gives a count of 24 fibers, we can use Figure 2 to determine that the mean inter-laboratory 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 liter volume, then the measured concentration is 0.1 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, it is estimated that there is a 90% probability that the mean would fall between 0.048 and 0.327 fiber/mL. It is recommended that these limits be reported in any comparison of results between laboratories (see Step 21).

Note that the s_r of 0.45 used to derive Figure 2 is used as an estimate for a random group of laboratories. If several laboratories belonging to a quality assurance group can show that their inter-laboratory 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 for certain types of samples, such as asbestos cement, that the s_r can be higher. This should be taken into account when comparing results for these types of samples.

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 2 indicates that the 0.5 fibers/mL standard must be 213% higher than the measured air concentration. This indicates that if one measures a fiber concentration of 0.16 fibers/cc (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.

$$[0.16 + (2.13 \times 0.16) = 0.5].$$

It can be seen from Figure 2 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. 213% and -49% that are obtained for a 100-fiber count.

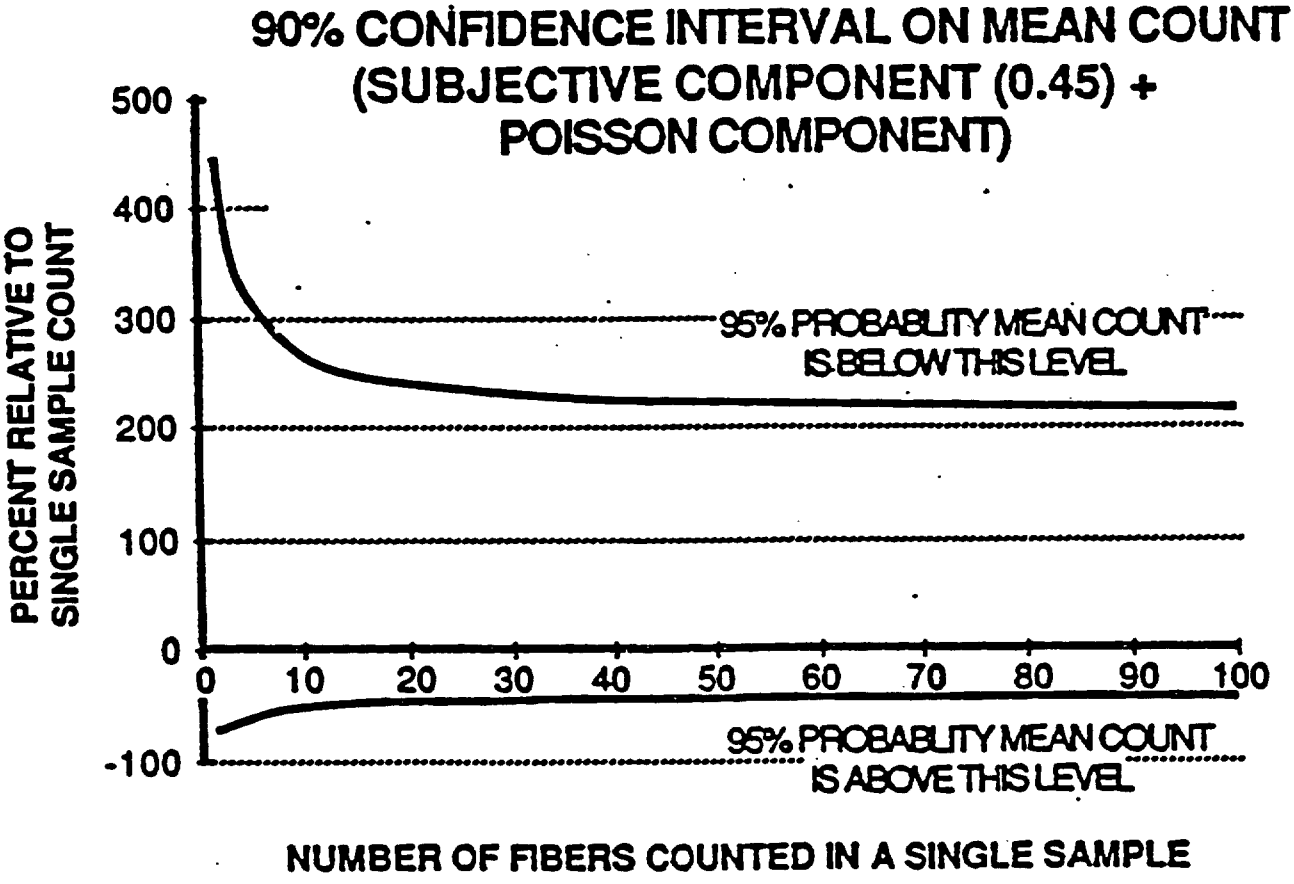

Figure 2.