Standard Practice for
Sampling and Counting Airborne Fibers, Including Asbestos Fibers, in Mines and Quarries, by Phase Contrast Microscopy and Transmission Electron Microscopy[1]

1. Scope

1.1 This practice[2] describes the determination of the concentration of fibers, expressed as the number of such fibers per millilitre of air, using phase contrast microscopy and optionally transmission electron microscopy to evaluate particulate material collected on a membrane filter in the breathing zone of an individual or by area sampling in a specific location. This practice is based on the core procedures provided in the International Organization for Standardization (ISO) Standard ISO 8672(**1**)[3], the National Institute for Occupational and Health (NIOSH) Manual of Analytical Methods, NIOSH 7400 (**2**), and the Occupational Safety and Health Administration (OSHA) Method ID 160 (**3**). This practice indicates the important points where these methods differ, and provides information regarding the differences. However, selecting portions of procedures from different published methods generally requires a user to report that they have used a modification to a method rather than claim they have used the method as written. This practice further gives guidance on how differential counting techniques may be used to indicate where a population of fibers may be asbestos.

1.2 The practice is used for routine determination of an index of occupational exposure to airborne fibers in mines, quarries, or other locations where ore may be processed or handled. The method gives an index of airborne fiber concentration. The method provides an estimate of the fraction of counted fibers that may be asbestos. This practice should be used in conjunction with electron microscopy (See Appendix X1) for assistance in identification of fibers.

1.3 This practice specifies the equipment and procedures for sampling the atmosphere in the breathing zone of an individual and for determining the number of fibers accumulated on a filter membrane during the course of an appropriately-selected sampling period. The method may also be used to sample the atmosphere in a specific location in a mine or in a room of a building (area sampling).

1.4 The ideal working range of this practice extends from 100 fibers/mm^2 to 1300 fibers/mm^2 of filter area. For a 1000-L air sample, this corresponds to a concentration range from approximately 0.04 to 0.5 fiber/mL (or fiber/cm^3). Lower and higher ranges of fiber concentration can be measured by reducing or increasing the volume of air collected. However, when this practice is applied to personal sampling in mines and quarries, the level of total suspended particulate may impose an upper limit to the volume of air that can be sampled if the filters produced are to be of appropriate particulate loading for fiber counting.

1.5 Users should determine their own limit of detection using the procedure in Practice D6620. For reference, the NIOSH 7400 method gives the limit of detection as 7 fibers/mm^2 of filter area. For a 1000-L air sample, this corresponds to a limit of detection of 0.0027 fiber/mL (or fiber/cm^3). For OSHA ID 160 the limit of detection is given as 5.5 fibers/mm^2 of filter area. For a 1000-L air sample, this corresponds to a limit of detection of 0.0022 fiber/mL (or fiber/cm^3).

1.6 If this practice yields a fiber concentration that does not exceed one-half the permissible exposure limit or threshold limit value for the particular regulated fiber variety, no further action may be necessary. If the fiber concentration exceeds one-half of the regulated permissible exposure limit or threshold limit value for the particular regulated fiber variety, it is necessary to examine the data to determine if more than 50 % of the counted fibers are thinner than 1.0 µm, or thicker but with an appearance of asbestos (curvature, splayed ends, or the appearance of a bundle).

1.7 The mounting medium used in this practice has a refractive index of approximately 1.45. Fibers with refractive indices in the range of 1.4 to 1.5 will exhibit reduced contrast, and may be difficult to detect.

[2] This test method is based on NIOSH 7400, OSHA Method ID 60, and ISO 8672. Users of this ASTM standard are cautioned that if they wish to comply with one of these specific procedures exactly they should follow that procedure, otherwise they should document the modification.
[3] Boldface numbers in parentheses refer to the list of references appended to this method.

1.8 Fibers less than approximately 0.2 µm in diameter may not be detected by this practice. (**4**)

1.9 *This standard may involve hazardous materials, operations, and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. For specific precautionary statements, see Section* 7.

2. Referenced Documents

2.1 *ASTM Standards:*[4]

D257 Test Methods for DC Resistance or Conductance of Insulating Materials

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

D1357 Practice for Planning the Sampling of the Ambient Atmosphere

D3670 Guide for Determination of Precision and Bias of Methods of Committee D22

D5337 Practice for Flow Rate Adjustment of Personal Sampling Pumps

D6620 Practice for Asbestos Detection Limit Based on Counts

2.2 *Other Standards:*

NIOSH 7400 National Institute of Occupational Health and Safety (Revised 1994)[5]

(RTM 1) Asbestos International Association (AIA), Recommended Technical Method No. 1[6]

ID 160 Occupational Safety and Health Administration (OSHA)

ISO 8672 International Organization for Standardization

3. Terminology

3.1 Description of terms specific to this practice, in addition to those found in Terminology D1356:

3.1.1 *asbestos*—a term applied to six specific silicate minerals belonging to the serpentine and amphibole groups, which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong fibers when crushed or processed (**5**). The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), riebeckite asbestos (crocidolite) (12001-28-4), grunerite asbestos (Amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4).

The precise chemical composition of each species varies with the location from which it was mined. Other amphibole minerals which exhibit the characteristics of asbestos have also

been observed (**6**).

The nominal compositions of the most common asbestos varieties are:

Chrysotile	$Mg_3Si_2O_5(OH)_4$
Crocidolite	$Na_2 Fe_3^{2+}Fe_2^{3+} Si_8O_{22}(OH)_2$
Amosite	$(Mg,Fe)_7 Si_8O_{22} (OH)_2$
Anthophyllite	$(Mg,Fe)_7 Si_8O_{22} (OH)_2$
Tremolite	$Ca_2(Mg,Fe)_5 Si_8O_{22} (OH)_2$ [Mg/(Mg + Fe^{2+}) 0.9 − 1.0]
Actinolite	$Ca_2(Mg,Fe)_5 Si_8O_{22} (OH)_2$ [Mg/(Mg + Fe^{2+}) 0.5 − 0.9]

NOTE 1—Actinolite compositions in which $Mg/(Mg + Fe^{2+})$ is between 0 and 0.5 are referred to as ferroactinolite. See Ref. (**7**) for the full naming conventions specified by the International Mineralogical Association.

3.1.2 *area sample*—an air sample collected so as to represent the concentration of airborne dust in a specific mine location, or area, or room of a workplace.

3.1.3 *asbestiform*—a specific type of fibrous mineral growth habit in which the fibers and fibrils exhibit a polyfilamentous growth habit and possess high tensile strength and flexibility. All materials regulated as asbestos are asbestiform, but not all asbestiform minerals are classified as asbestos. Characteristics such as tensile strength and flexibility cannot be ascertained from microscopic evaluation.

3.1.4 *asbestos fiber*—a fiber of asbestos that meets the criteria specified below for "fiber." Phase Contrast Microscopy (PCM) does not identify fibers unequivocally as asbestos. Under the light microscope, a population of asbestos fibers may appear as a mixture of fiber agglomerates, fiber bundles (polyfilamentous growth, unique to asbestiform fibers) fibers with split ends, and single fibers, the relative occurrence and frequency of each type depending on the situation.

3.1.5 *aspect ratio*—the ratio of the length of a fiber to its width.

3.1.6 *cleavage fragments*—mineral particles, normally formed by comminution of minerals, which often are characterized by parallel sides and a moderate aspect ratio (usually less than 20:1). Non-asbestiform cleavage fragments do not exhibit fibrillar bundling at any level of examination.

3.1.7 *limit of detection*—the number of fibers necessary to be 95 % confident that the result is greater than zero.

3.1.8 *differential counting*—a term applied to the practice of excluding certain kinds of fibers from the fiber count because they do not appear to be morphologically consistent with fibers of a specific variety thus modifying the definition of fiber given below.

3.1.9 *fiber*—an elongated particle that is longer than 5.0 µm, with a minimum aspect ratio of 3:1, and sometimes also classified as having a maximum width of 3.0 µm as this latter dimension may equate to the size of fiber of the density of many silicate minerals capable of penetrating the lung. An asbestos fiber should further exhibit the asbestiform habit, although analysis of airborne fibers by PCM may not be sufficient in itself to determine asbestiform habit.

3.1.10 *fibril*—a single fiber of asbestos that cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances.

3.1.11 *fibrous*—a mineral that is composed of parallel, radiating, or interlaced aggregates of fibers, from which the

---

[4] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For Annual Book of ASTM Standards volume information, refer to the standard's Document Summary page on the ASTM website.

[5] This standard is available from US Department of Health and Human Services, 4676 Columbia Parkway, Cincinnati, OH 45226.

[6] Available from Asbestos International Association, 68 Gloucester Place, London, W1H 3HL, England.

fibers are sometimes separable. A crystalline aggregate may be referred to as fibrous even if it is not composed of separable fibers, but has that distinct appearance. The term "fibrous" in mineralogy is used to describe aggregates of grains that crystallize in a needle-like habit and appear to be composed of fibers. Asbestos minerals are fibrous, exhibiting a specific type of fibrous habit termed asbestiform. However, not all minerals having fibrous habit are asbestos.

3.1.12 *field (of view)*—the area within the graticule circle that is superimposed on the microscope image.

3.1.13 *habit*—the characteristic crystal growth form or combination of these forms of a mineral, including characteristic irregularities.

3.1.14 *personal sample*—a sample taken by a collection apparatus (membrane filter) positioned in the breathing zone of the subject (near the nose and mouth) such that the collected particles are representative of airborne dust that is likely to enter the respiratory system of the subject in the absence of respiratory protection.

3.1.15 *set*—a group of samples that are collected, submitted to the laboratory, and analyzed for a report that is generated.

3.1.16 *RIB Graticule*—an eyepiece graticule specifically designed for asbestos fiber counting. It consists of a circle with a nominal projected diameter of 100 μm (nominal area of 0.00785 mm^2) with a cross-hair having dash lines 5-μm long and 0.5-μm wide in one direction and 5-μm long and 1-μm wide in the orthogonal direction. There are also examples around the periphery of the circle to illustrate specific sizes and shapes of fibers. This design of the graticule is shown in Fig. A1.1. The graticule is placed in one of the microscope eyepieces so that the design is superimposed on the field of view.

3.1.17 *Walton Beckett Graticule*—an eyepiece graticule specifically designed for asbestos fiber counting. It consists of a circle with a nominal projected diameter of 100 μm (nominal area of 0.00785 mm^2) with a cross-hair having tick-marks at 3 μm intervals in one direction and 5 μm intervals in the orthogonal direction. There are also examples around the periphery of the circle to illustrate specific sizes and shapes of fibers. This design of the graticule is shown in Fig. A1.2. The graticule is placed in one of the microscope eyepieces so that the design is superimposed on the field of view.

3.1.18 *HSE/NPL*[7] test slide—a calibration slide designed to determine the limit of visibility of a PCM and an observer.

4. Summary of Practice

4.1 The sample is collected by drawing air through a 25-mm diameter, mixed cellulose ester (MCE) membrane filter, housed in a conductive polypropylene cassette. After sampling, a sector of the membrane filter is converted to an optically transparent homogeneous gel. Fibers longer than 5 μm are counted by observing them with a Phase Contrast Microscope (PCM) at a magnification between 400 and 500.

4.2 Divide the total count into three groups: 1) bundles, fibers with splayed ends, or fibers with curvature; 2) fibers longer than 10 μm or fibers thinner than 1.0 μm; 3) all other fibers longer than 5 μm with a minimum aspect ratio of 3:1.

4.3 If 50 % or more of the total fiber count falls into Group 1 or 2, then there is an indication that asbestos fibers are present in the sample. It is recommended that the sample be evaluated using transmission electron microscopy (Appendix X1) to confirm the presence of asbestos and verify the asbestos concentration.

5. Significance and Use

5.1 Users of this practice must determine for themselves whether the practices described meet the requirements of local or national authorities regulating asbestos or other fibrous hazards.

5.2 Variations of this practice have been described by the Asbestos Research Council in Great Britain (**8**), the Asbestos International Association (AIA) (RTM 1) (**9**), NIOSH 7400, OSHA (Reference Method ID 160), and ISO 8672. Where the counting rules of these methods differ, this is noted in the text.

5.3 *Advantages:*

5.3.1 The technique is specific for fibers. PCM is a fiber counting technique that excludes non-fibrous particles from the analysis.

5.3.2 The technique is inexpensive, but requires specialized knowledge to carry out the analysis for total fiber counts, at least in so far as the analyst is often required under regulations to have taken a specific training course (for example, NIOSH 582, or equivalent).

5.3.3 The analysis is quick and can be performed on-site for rapid determination of the concentrations of airborne fibers.

5.3.4 The procedure provides for a discriminate counting technique that can be used to estimate the percentage of counted fibers that may be asbestos.

5.4 *Limitations:*

5.4.1 The main limitation of PCM is that fibers are not identified. All fibers within the specified dimensional range are counted. Differential fiber counting may sometimes be used to discriminate between asbestos fibers and fibers of obviously different morphology, such as cellulose and glass fiber. In most situations, differential fiber counting cannot be used to adequately differentiate asbestos from non-asbestos fibers for purposes of compliance with regulations without additional positive identification. If positive identification of asbestos is required, this must be performed by polarized light or electron microscopy techniques, using a different portion of the filter.

5.4.2 A further limitation is that the smallest fibers visible by PCM are about 0.2 μm in diameter, while the finest asbestos fibers may be as small as 0.02 μm in diameter.

5.4.3 Where calculation of fiber concentration provides a result exceeding the regulatory standard, non-compliance is assumed unless it can be proven that the fibers counted do not belong to a member or members of the group of fibers regulated by that standard.

---

[7] Health and Safety Executive/National Physical Laboratory – United Kingdom.

6. Interferences

6.1 If the practice is used to monitor a specific type of fiber, any other airborne fibers present will interfere since all particles meeting the counting criteria are counted. Some common fibers, which often create interference, are: gypsum, plant fibers, cellulose, perlite veins, diatoms, cellular plastic, mold mycelium, and cleavage fragments of minerals.

6.2 Particle aggregates consisting of chains of small particles, such as smoke or welding fume, may be perceived to be fibers and give rise to elevated results.

6.3 Platy particles such as talc sometimes can be oriented with the flat side perpendicular to the plane of the filter, and in this orientation they may appear as countable fibers.

6.4 High levels of non-fibrous dust particles may obscure fibers in the field of view and decrease the measured concentration, seriously underestimating the actual exposure.

6.5 Some membrane filters have inhomogeneous regions in which the polymers are not properly mixed, and the clearing process gives rise to features that cannot be distinguished from fibers.

6.6 Cleavage fragments of non-asbestiform anthophyllite, tremolite, actinolite and other minerals that have dimensions within the range of the fiber definition in this practice will be included in the fiber counting result.

7. Precautions

7.1 Asbestos is a known carcinogen; as such proper ventilation must be provided during the sample handling so as to minimize the risk of inhalation by the technician during sample preparation if the asbestos is suspected to be present in a form that may become airborne. Handle the samples in such a way as to avoid causing the fibers to become an airborne inhalation hazard. To minimize the risk of inhalation by the analyst during sample preparation in the laboratory, the exterior of cassettes should be cleaned of visible dust and debris in the field before they are submitted. Consult the materials safety data sheets for asbestos and all reagents listed in Section 9.

8. Apparatus

8.1 *Sampler, field monitor*—25-mm diameter, three-piece conductive[8] cassette with 50-mm extension cowl and with a mixed cellulose ester filter, 0.45 to 1.2 μm pore size, supported by a back-up pad. Wrap the joint between the extension piece and cassette base with a shrink seal or tape to prevent dust from lodging in the joints. If other cassettes are used, they must be evaluated to ensure that the percentage of fiber losses to the walls does not exceed those of the standard cassette, and that the uniformity of deposition across the filter is equivalent or better than that of the standard cassette.

Note 2—Analyze representative filters for fiber background before use and reject the cassette lot if the average result is more than 5 fibers/100

fields. Pre-counting of filters by the manufacturer may be accepted if confirmed by field blanks being within acceptable range.

Note 3—Cassettes shall not be re-used or re-loaded.

8.2 *Sampling Pump*—For personal sampling, a battery powered pump, minimum flow rate 0.5 L/min (see 10.3 for discussion on flow rate), with flexible connecting tubing. For area sampling, a supply powered pump, capable of flow rates up to 16.0 L/min may be used in place of personal sampling pumps when higher flow-rates or longer sampling times are desired.

8.3 *Microscope,* positive phase (dark) contrast with a binocular or trinocular head, green or blue filter, adjustable field iris, wide-field or Huygenian 8× to 10× eyepieces, and a 40× to 45× phase objective with numerical aperture 0.65 to 0.75, to give a total magnification of approximately 400 to 450.

Note 4—The eyepiece containing the graticule must be a focusing eyepiece.

Note 5—Köhler illumination should be used, if possible.

8.4 *Microscope Slides, glass,* pre-cleaned, 25 mm by 75 mm. Slides with one end frosted are convenient for labeling using a pencil, or alternatively, adhesive labels may be used.

8.5 *Cover Slips*—22 mm by 22 mm, No. 12, unless another thickness of cover slip is specified by the manufacturer of the objective lens.

8.6 *Scalpel Holder and Disposable Blades*—#10 or #22 surgical steel, curved blade.

8.7 *Forceps*—Fine point.

8.8 *Acetone Vaporizer,* a device used to clear MCE filters by exposure to a small amount of vaporized acetone.

8.9 *Syringe, Hypodermic,* with 22-gauge needle or disposable micropipet.

8.10 *Graticule, either Walton Beckett or RIB,* with 100 μm diameter circular filed at the plane of the specimen. The designs of the graticules are shown in Fig. A1.1 and Fig. A1.2.

Note 6—The graticule is custom-made for each microscope such that the diameter of the circular field is 100 μm at the plane of the specimen. It is necessary to specify the disc diameter needed to fit the ocular of the microscope and the diameter (mm) of the circular counting area (see Annex A1).

8.11 *HSE/NPL*[7] Phase Contrast Test Slide—Mark II.[9]

8.12 *Telescope Ocular*—Used for viewing and centering phase-rings.

8.13 *Stage Micrometer*—1 mm divided into 0.01 mm divisions.

9. Reagents

9.1 *Acetone,* reagent grade

9.2 *Triacetin (glycerol triacetate),* reagent grade.

9.3 *Lacquer or Nail Polish.*

---

[8] "Conductive" as defined by ESD Association, 790 Turin Rd., Suite 4, Rome, NY 13440, as having a resistance of less than 1×10^5 ohms per square surface. Conductance as measured by Test Methods D257. Conductivity should be assured by the manufacturer.

[9] The sole source of supply of the apparatus known to the committee at this time is Optometrics USA., Nemco Way, Ayer MA, 01432. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

10. Sampling

10.1 See Practice D1357 for guidance on sampling procedures.

10.2 Calibrate each personal sampling pump with a representative cassette in line. Connect each pump to a calibration cassette with an appropriate length of 6 mm (0.25 in.) bore plastic tubing.

NOTE 7—This calibration cassette should be from the same lot of cassettes used for sampling.

10.3 Select an appropriate flow rate for the situation being monitored. The sampling flow rate must be between 0.5 and 5.0 L/min for personal sampling and is commonly set between 1 and 2 L/min. For area sampling, a flow rate of up to a maximum of 16.0 L/min may be used.

NOTE 8—Do not overload the filter with dust. High levels of non-fibrous dust particles may obscure fibers on the filter and lower the count or make counting impossible. If more than about 25 to 30 % of the field area is obscured with dust, the result may be biased low. Smaller air volumes may be necessary when there is excessive non-asbestos dust in the air (but a minimum of 25 L should be taken). While sampling, observe the filter with a small flashlight. If there is a visible layer of dust on the filter, stop sampling, remove and seal the cassette, and replace with a new sampling assembly.

10.4 Calibrate sampling pumps before and after sampling with a calibration cassette in-line, as close as possible to the sampling site, using the procedures described in Practice D5337. Note that a primary flow-rate calibrator can only be considered a primary standard for calibration purposes if the calibration is traceable to national standards (see Practice D5337).

NOTE 9—If calibration at the sampling site is not possible, environmental influences may affect the flow rate. The extent is dependent on the type of pump used. Consult the pump manufacturer to determine dependence on environmental influences. If the pump is affected by temperature and pressure changes, use the formula provided by the manufacturer to calculate the actual flow rate.

10.5 Fasten the plastic tubing with spring clips or similar devices on the worker's lapel or other part of the worker's clothing so that the sampler is as close as possible to the worker's mouth. Remove the plug from the outlet end of the cassette before starting the pump to avoid creating a vacuum inside the cassette and then slowly remove the top cover from the end of the cowl extension (open face) and orient the cassette face down. Ensure that each sample cassette is held open side down in the employee's breathing zone during sampling.

10.6 The user must determine the blank level on the filters to be used. As a guide to acceptability, the NIOSH 7400 requires quality control on laboratory blank filters to be less than 5 fibers/100 fields (manufacturer's test results are acceptable). Each set of samples taken will include 10 % field blanks or a minimum of two field blanks. These blanks must come from the same lot as the filters used for sample collection. Remove and replace the caps from the field blank cassettes and store the cassettes in a clean area (bag or box) during the sampling period. Replace the caps in the cassettes when sampling is completed. Average the field blank results and subtract from the analytical results before reporting, if re-

quired. Both NIOSH 7400 and OSHA ID 160 require field blank results to be subtracted from the final result, but NIOSH 7400 requires the reporting of field blank levels greater than 7 fibers/100 fields due to possible contamination. In other cases, blanks may not be required to be subtracted from the final result. However, field blank results must always be reported and whether they have been subtracted from sample results. A set consists of any sample or group of samples for which an evaluation for this practice must be made.

10.7 Sample at 0.5 L/min or greater ([10]). Collect a minimum volume of 25 litres for personal samples. For optimum counting precision, adjust the sampling flow rate, Q, and time, t, to produce a fiber density, E, of 100 fibers/mm^2 to 1300 fibers/mm^2 (3.85×10^4 to 5×10^5 fibers on a 25-mm filter with an effective collection area A_c of 385 mm^2).

$$t_{min} = \frac{A_c E}{1000 Q L} \qquad (1)$$

where:

t_{min} = minimum sampling time, minutes
A_c = effective collection area, mm^2
E = fiber density, fibers/mm^2
Q = sampling flow rate, L/min
L = half the minimum concentration of interest to the user of this practice, fibers/mL (or fibers/cm^3)
1000 = conversion factor, mL (or cm^3) to L.

NOTE 10—Periodically check and adjust the value of A_c due to variations, from manufacturer to manufacturer, of the effective collection area of cassettes.

10.8 Remove the field monitor at the end of sampling, replace the plastic top cover and small end caps, and store the monitor.

NOTE 11—Do not replace the cap and plug before removing the cassette from an operating pump. This will cause a vacuum in the cassette and rupture the filter, possibly displacing collected particulate from the filter and destroying the value of any filter analysis.

10.9 If samples are shipped, it is recommended that samples are packed in a rigid container with sufficient static dissipative foam packing material to prevent jostling or damage. Certain static-causing materials are prohibited by regulation, including paper and non-dissipative polystyrene packing materials ([11, 12]).

11. Sample Preparation

11.1 Wipe the exterior of the sampling cassettes with a damp cloth to minimize the possibility of contaminating the filter after opening the cassette.

11.2 Ensure that the glass slides and cover slips to be used are free of dust and fibers.

NOTE 12—The objective is to produce samples with a transparent, smooth (non-grainy) background in a medium with a refractive index equal to or less than 1.46. The method described below collapses the filter for easier focusing and produces permanent mounts, which can be stored for quality control and inter laboratory comparisons. Alternative slide preparation techniques may also be used ([13]).

11.3 Remove the top plug to prevent development of a vacuum when the cassette is opened. Using a scalpel or razor blade, cut the seal, or tape around the cassette, or both at the

depression between the base and the extension cowl. Very carefully separate the base from the extension cowl, leaving the filter and back-up pad in the base. To avoid possible contamination of the filter, do not use the same scalpel that will be used to cut the filter.

11.4 Using forceps, grasp the filter at the perimeter of the filter that was clamped between the cassette pieces. To do this without damage to the filter, it is helpful to use a long, thin object to push the back-up pad/filter pair above the base of the cassette so the filter can be grasped from the edge. DO NOT TOUCH the surface of the filter. Place the filter on a clean glass slide with the deposit side facing upwards. A fresh slide should be used each time to eliminate possible cross-contamination. An alternative approach that achieves the same aim is to cut a wedge from the filter as it lies within the cassette.

11.5 Carefully examine the filter. Reject the filter if it does not exhibit a complete circular impression made by the edge of the extension cowl, or if sharp edges on the extension cowl have cut through the filter. Observation of either situation is cause to reject the filter as defective, because leakage around the edge of the filter in an improperly clamped cassette, or leakage through the cut areas of the filter, will lead to a negative bias in the result.

11.6 Cut 90° sectors of approximately 25 % of the filter area with a curved blade steel surgical scalpel (not the same one that was used to cut the perimeter band of the cassette). Place the filter or sector, dust side up, on a clean, labeled, microscope slide. Static electricity will usually keep the filter on the slide until it is cleared. Use care not to disturb the particles on the filter. Return the remainder of the filter to the cassette for storage.

Note 13—If preferred, the whole sample filter may be mounted and cleared. However, this will not leave any sample filter available for subsequent quality assurance measurements or optional examination by transmission electron microscopy.

11.7 Insert the slide into the acetone vaporizer, centering the filter sector under the delivery spout. Inject acetone in accordance with the manufacturer's instructions to clear the filter. Remove the slide from the vaporizer.

Note 14—Use a minimum volume of acetone. Excess acetone may flush fibers from the filter yielding low results. For most acetone vaporizers, a nominal volume between 100-250 μL is appropriate for each slide. Acetone is extremely flammable and precautions must be taken not to ignite it. Avoid using large containers or large quantities of acetone. Transfer the solvent in a ventilated laboratory hood. Do not use acetone near any open flame. For generation of acetone vapor, use a spark-free heat source.

11.8 Immediately (less than 30 seconds), using the hypodermic syringe with an approximately 22 gauge needle, or a micro-pipet with a disposable tip, place 1 to 2 drops of triacetin on the filter surface. Gently lower a clean approximately 22-mm square cover slip onto the filter at a slight angle to reduce the possibility of forming and trapping air bubbles between the cover slip and the filter. If too many bubbles form or the amount of triacetin is insufficient, or too much time passes between the acetone and application of triacetin, the cover slip may become detached within a few hours. Use only enough triacetin to cover the filter. More will spill over the edge allowing for later fiber migration, minimizing the value of the slide preparation for archival purposes.

Note 15—Allowing the slide to stand for longer than 30 seconds before the triacetin is added will result in an increased index of refraction of the mounting medium and consequently decreased contrast between the fibers and the preparation.

11.9 The clearing process is usually slow. The clearing process may be accelerated by warming the slide on a hotplate (surface temperature 50°C – 55°C) or in an oven at this temperature for 15 minutes.

11.10 For samples retained for quality assurance or archival purposes, seal the edges of the cover slip to the glass slide using a lacquer or nail polish (**9**). Samples with too much triacetin may be unsuitable for archival purposes, since the fibers may migrate.

Note 16—It is recommended that the outline of the active collection area of the filter sector be marked on the base of the slide, using a waterproof fiber-tipped pen, between the clearance and mounting steps. This ensures that fiber counting is confined to the active collection area, and provides a permanent reference after the edges of the filter sector have become difficult to see. Some movement of the particulate material will continue to occur during storage, but this movement is not sufficient to significantly affect the reported results.

12. Microscope Calibration

12.1 Ensure that all optical surfaces are clean. Even a small amount of dirt can significantly degrade the image.

12.2 *Microscope Adjustments*—Follow the manufacturer's instructions and also the following:

12.2.1 Adjust the light source for even illumination across the field of view at the condenser iris.

12.2.2 Focus the microscope on the particulate material to be examined.

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

12.2.4 Use the telescope ocular supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are accurately concentric. Critically center the rings. Misalignment of the rings will result in astigmatism and a degraded image.

12.3 *Testing of the Visibility Performance of the Microscope:*

12.3.1 Periodically check the phase-shift detection limit of the microscope using the HSE/NPL phase-contrast test slide, as described in Annex A2.

12.3.2 If the image quality deteriorates, clean the microscope optics, and if the problem persists, consult the microscope manufacturer.

12.4 Ensure the size of the graticule (see section Annex A1) is checked when received, and whenever the microscope is disassembled/reassembled, such as during maintenance or cleaning.

13. Measurement

13.1 Follow the alignment routine specified in Section 12 at the beginning of every counting session and more often if it is found to be necessary. In particular, check the alignment of the

phase rings before counting each sample, because any slight angle that may sometimes exist between the slide and the cover slip can result in misalignment of the phase rings. Report the use of the RIB graticules where this was done. No data currently exist on intercomparisons of the two graticules types. The ASTM committee responsible for this practice strongly encourages potential users of the RIB graticules to obtain such data and, if an acceptable intercomparison is not established within a reasonable period of time, it is the intention of the committee to ballot a revision to this practice to remove mention of this graticule.

13.2 Place the prepared sample slide on the mechanical stage of the microscope. Position the center of the filter sector under the objective lens and focus upon the sample.

13.3 Start the count from one end of the filter sector and progress along a radial line to the other end. The count may be performed in either direction from the perimeter to the tip of the filter sector. Select fields randomly, without looking into the eyepieces, by slightly advancing the slide in one direction with the mechanical stage control.

13.4 Select graticule fields at least 1 mm from the cut edges, and at least 1 mm into the deposit area at the filter periphery. A line drawn on the slide around the inside of the periphery of the filter can aid in meeting this requirement.

13.5 Using the fine focus control, continually scan over a range of focal planes (generally the upper 10 to 15 μm of the filter surface) during the examination of each graticule field. A minimum time of 15 seconds for examination of each graticule field is appropriate for accurate counting. Taking insufficient time will result in the analyst missing very fine fibers if they are present. This is a common counting error when chrysotile fibers are present. This is a systematic bias (error).

13.6 All asbestos types exhibit some asbestos fibers with diameters less than 1 μm. Examine each graticule field care-fully for faint fiber images. Small diameter fibers will be very hard to see. However, they are often an important contribution to the total count.

13.7 Count only fibers longer than 5 μm that also have length to width ratios of 3:1. Measure the length of curved fibers along the curve.

13.8 Count all the fibers in at least 20 fields subject to counting rules as in the table in Annex A3. Continue the count until either 100 fibers have been counted or 100 graticule fields have been examined, whichever occurs first. Count all of the fibers in the final graticule field.

13.9 Any fiber lying entirely within the boundary of the graticule field shall be counted as one. Any fiber crossing the boundary of the graticule field once, having one end within the circle, shall be counted as ½. If a fiber touches the circle of the graticule field, it is considered to cross the line and shall be counted as ½. Do not count any fibers that are totally outside the graticule area.

13.10 Count a bundle of fibers as one fiber unless the individual constituent fibers can be clearly identified and each individual constituent fiber is clearly not connected to another counted fiber.

13.11 Record the number of fibers in each graticule field in a consistent way, using a data sheet such as that shown in Fig. 1, so that any non-randomness in the filtered particulate can be evaluated. If a field has no fibers counted, it must be recorded as "0" and not left blank.

13.12 When an agglomerate (mass of material) or a bubble covers more than 25 % of the graticule field, reject the graticule field and select another. Do not include the rejected field in the number of graticule fields counted and always record such occurrences. If the percentage of rejected fields exceeds 25 % of the total number of fields, there is a possibility that the count may be biased low, and this situation should be reported. If the number of rejected fields is too large to allow sufficient fibers or fields to be counted in accordance with 13.8, then the sample should be rejected as overloaded.

13.13 *Differential Fiber Counting*—Separate the fiber counts into the following three groups as the fibers are counted. See Annex A4 for examples. When a population of fibers with dimensions consistent with Note 17 is observed, asbestos confirmation may be desirable by means of the methodology set out in Appendix X1.

Note 17—Asbestos fibers are typically <1 μm diameter unless present as bundles or clusters that exhibit the characteristic fibrillar structure of asbestiform minerals, regardless of length. Cleavage fragments greater than 5 μm in length are typically >1 μm diameter, and they do not exhibit the fibrillar structure of asbestos.

13.13.1 *Group 1*—Those fibers that are thinner than 1.0 μm.

13.13.2 *Group 2*—Those fibers thicker than 1.0 μm that show curvature or have splayed ends, or have the appearance of a bundle.

13.13.3 *Group 3*—All other countable fibers.

13.14 *Modification of PCM Data by Transmission Electron Microscopy (TEM)*—When a fiber count by PCM exceeds the limit value, and it is suspected that there is interference by unregulated fiber species, the PCM fiber count may be modi-fied by TEM measurement of the proportion of the regulated fibers in the PCM count. Follow the procedure described in Appendix X1.

14. Calculations

14.1 Calculate and report the fiber density on the filter, *E*, where

$$E = \frac{\dfrac{F}{n_f} - \dfrac{B}{n_b}}{A_f} \qquad (2)$$

where:
E = fiber density, fibers/mm^2,
F = fiber count,
B = mean field blank count,
n_f = number of graticule fields examined during counting of the sample,
n_b = mean number of graticule fields examined during counting of the field blanks,
A_f = actual field area (0.00785 mm^2 for a properly calibrated graticule)

PHASE CONTRAST MICROSCOPY FIBER COUNT
ASTM D 7200

Sample Number: _____ Date Received: _____

Sample: _____ Prepared By: _____ Date: _____

_____ Analyzed By: _____ Date: _____

_____ Air Volume: _____ Liters

Group	Counted Fields								
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									
1									
2									
3									

SAMPLE: _____ Fibers (F) _____ Fields (n_f) _____ Fields Rejected

FIELD BLANK: _____ Fibers (B) _____ Fields (n_b)

Field Area (A_f): _____ mm^2 Filter Active Collection Area (A_c): _____ mm^2

Rejected Fields

Fiber Density	Fiber Concentration
$$E,\ fibers/mm^2 = \dfrac{\dfrac{F}{n_f} - \dfrac{B}{n_b}}{A_f}$$	$$C,\ fibers/mL = \dfrac{EA_c}{1000\ V}$$

FIG. 1 Example of Suitable Laboratory Work Sheet for Recording of PCM Fiber Count

A more conservative approach is to calculate the concentration without blank correction, although, in such case, the blank values should still be reported.

14.2 Calculate the concentration, C, of fibers in the air volume sampled, V, using the effective collection area of the filter, A_c:

$$C = \frac{EA_c}{1000V} \qquad (3)$$

where:

A_c = nominally, 385 mm^2 for a 25-mm filter (this may vary from manufacturer to manufacturer and other factors);

C = concentration of fibers in the air volume sampled, fibers/mL (or fibers/cm^3)

V = volume of air sampled, L, and;

1000 = conversion factor, L to mL (or cm^3).

A more conservative approach is to calculate the concentration without blank correction, although, in such case, the blank values shall still be reported along with whether or not the blank correction was applied.

14.3 Report the results as fibers/mL (or fibers/cm^3). Use the number of significant figures appropriate for the accuracy of the measurement. If multiple analyses are performed on a sample, an average of the results shall be reported unless any of the results can be rejected for cause, for example, overloading or quality issues as stated in Section 17. State in the report that the result is an average.

14.4 Also report the number of fields counted and the number of fibers found. Report the area of the filter and the area of the Walton-Beckett or RIB graticules (and specify the use of the RIB graticules where that was the case) where these differ from the nominal values given in 14.1 and 14.2.

14.5 Report as well, sampling parameter information such as area or personal sample, air volume, field area, detection limit, upper and lower confidence limits, and counting rules used. When the procedure has been carried out in compliance with a specific method, the method should be reported. Differential counting must always be reported if used.

14.6 If the concentration exceeds one-half regulated permissible exposure limit or threshold limit value, calculate the fraction of counted fibers that are either fibers thinner than 1.0 µm (Group 1) or bundles or fibers with curvature or splayed ends (Group 2).

14.7 If the fraction of counts from Groups 1 and 2 together exceed one-half of the total count, the data indicates the possibility of an asbestos fiber population. Proceed with the TEM analysis shown in Appendix X1.

15. Sampling and Analytical Errors

15.1 *General:*

15.1.1 Errors introduced in the estimation of airborne asbestos dust include sampling and analytical errors, each of which has a systematic and random component. The application of standard and reproducible routine is the only way of controlling most of the many sources of error inherent in the membrane filter method. The following list describes some of the common sources of error.

15.2 *Systematic Errors:*

15.2.1 *Sampling:*

15.2.1.1 Flow rate

15.2.1.2 Sampling time

15.2.1.3 Non-representative or biased sampling

15.2.1.4 Contamination, accidental or deliberate

15.2.2 *Analytical:*

15.2.2.1 Effective filter area

15.2.2.2 Counting area

15.2.2.3 Filter mounting

15.2.2.4 Microscope and observers

15.2.2.5 Cross contamination

15.3 *Random Errors:*

15.3.1 *Sampling:*

15.3.1.1 Flow rate variability

15.3.1.2 Random fluctuations of the airborne dust cloud

15.3.2 *Analytical:*

15.3.2.1 Fiber distribution on the filter

15.3.2.2 Non-random deposition of dust on the filter leads to gross errors, the magnitude of which cannot be estimated. Twenty or more fields must be counted to ensure that minor divergence from randomness does not bias the result.

15.3.2.3 Poisson errors.

15.3.2.4 As only small samples of the fibers deposited on the filter are counted, errors arise in the estimation of the total number of fibers on the entire filter face. Theoretically, the Poisson distribution defines the variation in fiber counts resulting from viewing randomly selected counting fields on the filter. If a minimum of 100 fibers is counted, and if a Poisson distribution were appropriate to the counting results, the relative standard deviation of the fiber counts would be 10 %. It has been shown experimentally that the actual distribution of fiber counts can depart from that of Poisson, in which case the standard deviation may be greater.

15.4 *Limitations of the Membrane Filter Method and Presentation of Results:*

15.4.1 Using typical parameters of 1 L/min flow rate for 8 hours, and a minimum filter loading of 5.5 fibers/100 graticule areas, the theoretical limit of detection is 0.0045 fiber/mL (or fiber/cm^3). However, in many situations the level of background dust limits the air volume that can be collected, and the practical limit of detection may therefore be much higher.

15.4.2 It is generally accepted that blank, unused filters can frequently give a reading of several countable fibers per 100 graticule areas. These fibers may be unidentified contaminants on the filter, or artifacts from the clearing process which have the appearance of fibers.

15.4.3 It must be recognized that neither counting more fields nor increasing sampling duration overcomes the problem of background dust, when fibers are a minimum constituent in the overall dust cloud.

16. Precision and Bias

16.1 Since it has not been possible to prepare a quantitative standard with a known fiber density, a measurement of bias is not possible.

16.2 *Inter-laboratory Comparability for Total Fiber Count:*

16.2.1 An interlaboratory collaborative study involved six laboratories using prepared slides collected from operating mines and quarries, an asbestos abatement operation, and from PAT (AIHA) samples (**14**). The relative standard deviations (S_r) varied with fiber loading. The ranges are shown in Table 1. The precision was highly dependent on the type of fiber counted and the nature of the material from which the airborne asbestos was dispersed.

For the studies cited in Table 1, the coefficient of variation across all sample types for a fiber loading of 100 f/mm^2 was found to be about 0.43.

16.2.2 There is no independent means to determine the overall accuracy of this practice. 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 inter-laboratory variability, as well as showing how the results of this practice relate to the theoretically attainable counting precision and to measured intra- and inter-laboratory S_r. The precision of a PCM fiber count has been found to consist of two components: the Poisson variability and a subjective component.

NOTE 18—The following discussion does not include bias estimates and should not be taken to indicate that lightly loaded samples produce results as accurate as properly loaded ones.

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

$$S_r = \frac{1}{\sqrt{N}} \qquad (4)$$

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 (**15, 16, 17**).

An additional component of variability comes primarily from subjective inter-laboratory differences. In a study of ten counters in a continuing sample exchange program, Ogden (**18**) found this subjective component of intra-laboratory S_r to be approximately 0.2 and estimated the overall S_r by the term:

$$S_r = \frac{\sqrt{N + (0.2N)^2}}{N} \qquad (5)$$

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 control sample. For laboratories not engaged in an intensive quality assurance program, the subjective component of variability can be higher.

16.2.4 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 (**19**). These results gave a subjective inter-laboratory component of S_r (on the same basis as Ogden's) for field samples of ca. 0.45. 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 American Industrial Hygiene Association Proficiency Analytical Testing (AIHA PAT) program for laboratory-generated samples (**15**).

16.2.5 A number of factors influence S_r for a given laboratory, such as the actual counting performance of the laboratory and the type of samples being analyzed. In the absence of other information from an inter-laboratory quality assurance program using field samples, the value for the subjective component of variability should be taken as 0.45. It is recommended that each laboratory establish an inter-laboratory quality assurance program to improve performance and thus reduce the S_r.

16.2.6 The intra-microscopist and inter-microscopist precision of differential fiber counting has been established based on the use of Group 1 identification alone and is within the range of precision for other aspects of this counting procedure. The addition of the Group 2 identification dramatically increases the inter-laboratory standard deviation because of gross differences in the recognition of Group 2 features by different microscopists. It can be argued from the published data (**20**) that Group 1 may be sufficient on its own for the purposes of this Standard. However, the Standard as written allows for the assured addition of asbestos particles thicker than 1.0 μm where present and, if non-asbestos particles are also included, it will only add to the likelihood of a conservative decision to require further examination.

16.3 *Presentation of Results*—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 about the mean count from a single sample fiber count (Fig. 2). These curves assume similar shapes for the count distributions for both inter-laboratory and intra-laboratory results (**18**). As an example, if a sample yields a count of 24 fibers, Fig. 2 indicates that the mean inter-laboratory count will fall within the range of 227 % above and 52 % below that value 90 % of the time. These percentages may also be applied directly to the air concentrations. If, for instance, this sample (24 fibers counted) represented a 500-L volume, then the measured concentration is 0.02 fiber/mL (or fiber/cm^3) (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 (or fiber/cm^3). These limits should be reported in any comparison of results between laboratories.

Note that the S_r of 0.45 used to derive Fig. 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 shall 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 (**21, 22**).

TABLE 1 Coefficients of Variation Observed For Fiber Counting of Slides Prepared From Filters Collected in Various Industries (14)

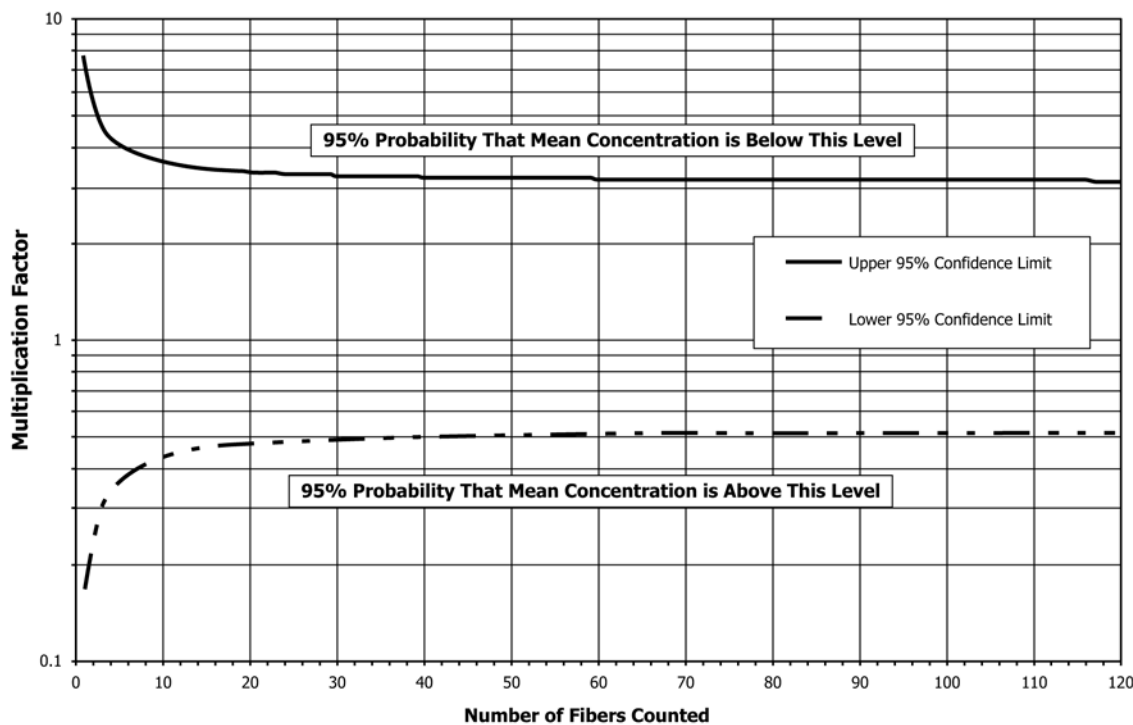| Fiber Loading	50 f/mm^2	200 f/mm^2	400 f/mm^2
Coefficient of Variation (CV)	0.53	0.36	0.29

FIG. 2 Inter-laboratory Precision of Fiber Counts

Fig. 1 is derived using the following equations:

$$UCL = \frac{2X + 2.25 + \sqrt{(2.25 + 2X)^2 - 4(1 - 2.25S_r^2)X^2}}{2X(1 - 2.25S_r^2)} \qquad (6)$$

$$LCL = \frac{2X + 4 - \sqrt{(4 + 2X)^2 - 4(1 - 4S_r^2)X^2}}{2X(1 - 4S_r^2)} \qquad (7)$$

where:

S_r = the subjective component of the inter-laboratory coefficient of variation;
X = the total number of fibers counted;
UCL = multiplication factor to obtain the upper 95 % confidence limit, and;
LCL = multiplication factor to obtain the lower 95 % confidence limit.

A group of laboratories that has collected sufficient data to derive its own value of S_r can use the formulae to derive 95 % confidence limit curves for use by the group.

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 a 0.5 fiber/mL (or fiber/cm^3) standard using a single sample on which 100 fibers have been counted, then Fig. 2 indicates that the measured air concentration must be a factor of 3.13 lower than the 0.5 fiber/mL (or fiber/cm^3) standard. This indicates that if a fiber concentration of 0.16 fiber/mL (or fiber/cm^3) (100 fibers counted) is measured, 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 fiber/mL (or fiber/cm^3); that is, 0.16 × 3.13 = 0.5.

It can be seen from Fig. 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 a factor of +3.13 and a factor of −0.49 as the upper and lower confidence values of the mean for a 100 fiber count.

17. Quality Assurance

17.1 *Quality Control of Fiber Counts*—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 19—The identity of the blank filters should be unknown to the counter until all counts have been completed.
NOTE 20—If a field blank yields fiber counts greater than 7 fibers/100 fields, report possible contamination of the samples.

17.2 Perform a "blind re-count" of 1 in every 10 filter sectors (slides). A person other than the original counter shall re-label the slides prior to the second examination.

17.3 Use the following test to determine whether a pair of counts 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 (X) $S_r^{'}$, where X = average of the square root of the two fiber counts and $S_r^{'}$ = half the intracounter relative standard deviation, which should be derived by each laboratory based on historical in-house data.

NOTE 21—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.
NOTE 22—Counts should be in fibers/mm^2

17.4 Analysts should also participate in a national sample testing scheme, such as the Asbestos Analyst Registry (AAR)

sponsored by the American Industrial Hygiene Association (AIHA), especially when working on site, instead of in a laboratory with an extensive quality assurance program. Laboratories should (and laboratories accredited by the AIHA for fiber-counting analyses are required to) participate in an appropriate scheme such as the Proficiency Analytical testing scheme sponsored by the AIHA. OSHA regulations also require laboratories to participate in round-robin testing with at least two other laboratories using real-world samples (AAR and AIHA PAT samples do not meet this requirement) (**12**). AIHA accreditation of laboratories also requires this additional round-robin activity.

17.5 Each analyst shall select and count prepared slides from a "slide bank." These are quality assurance counts. The

slide bank shall be prepared using uniformly distributed samples taken from the workload. Fiber densities should cover the entire range routinely analyzed by the laboratory. These slides are counted blind by all counters to establish an original standard deviation. This historical distribution is compared with the quality assurance counts. A counter must have 95 % of all quality control samples counted within three standard deviations of the historical mean. This count is then integrated into a new historical mean and standard deviation for the slide.

18. Keywords

18.1 air monitoring; asbestos; fibers; membrane filter method; mines and quarries; phase contrast microscopy; sampling and analysis

---

ANNEXES

(Mandatory Information)

A1. SPECIFICATION AND CALIBRATION OF EYEPIECE GRATICULE

A1.1 *The RIB Graticule*—The RIB eyepiece graticule is designed specifically for fiber counting. (**14**) A drawing of the graticule is shown in Fig. A1.1.

A1.1.1 The diameter of the RIB graticule is 100 µm ± 2 µm.

A1.1.2 Five circles with inscribed dots are located in the interior of the graticule diameter. Each circle has an apparent diameter of 5 µm. The dots are 1 µm in diameter.

A1.1.3 The vertical intermittent line is comprised of dashes that are 5-µm long and 0.5-µm wide. The space between each dash (excluding the end spaces) is 1 µm. The space from the center circle to the first dash is 3 µm.

A1.1.4 The horizontal intermittent line is comprised of dashes that are 5-µm long and 1-µm wide. The space between each dash (excluding the end spaces) is 1 µm. The space from the center circle to the first dash is 3 µm.

A1.1.5 Outside of the graticule circle, the 3:1 and 5:1 aspect ratio lines are each 5-µm long.

A1.1.6 Outside of the graticule circle are two 20:1 and 10:1 aspect ratio lines: one is 0.5 µm in width, the other 1-µm wide.

A1.1.7 Each aspect ratio outside of the graticule circle shows three dots. The diameters of these dots are 0.5, 0.75, and 1 µm.

A1.1.8 The equivalency of fiber counts obtained with the RIB graticule, compared with the Walton-Beckett graticule, has not yet been determined.

A1.2 *The Walton-Beckett Eyepiece Graticule*—The Walton-Beckett eyepiece graticule is designed specifically for fiber counting. A drawing of the graticule is shown in Fig. A1.2. The aspect ratios of this example are specific to the "A" counting rules.

A1.3 Specification of Eyepiece Graticule

A1.3.1 *Specification of Disc Diameter*—When ordering the graticule, specify the appropriate disc diameter for the internal diameter of the eyepiece into which it will be installed.

A1.3.2 *Determination of the Diameter of the Circular Counting Area for the Graticule:*

A1.3.2.1 Insert any available graticule into the eyepiece and focus the microscope so that the graticule lines are sharply in focus.

A1.3.2.2 Set the appropriate inter-pupillary distance and, if applicable, re-set the binocular head adjustment so that the magnification remains constant.

A1.3.2.3 Insert the 40× to 45× phase objective.

A1.3.2.4 Place the stage micrometer on the microscope object stage and focus the microscope onto the graduated lines.

A1.3.2.5 Measure the magnified length, L_o of a line on the graticule, using the stage micrometer.

A1.3.2.6 Remove the graticule from the microscope and measure the actual length, L_a (mm) of the selected line on the graticule. This can best be accomplished using a microscope stage fitted with vernier scales.

A1.3.2.7 Calculate the required diameter of the circular counting area for the graticule, d_c, for the graticule:

$$d_c = \frac{L_a}{L_o} \times D \qquad (A1.1)$$

where:

d_c = circle diameter to be specified, mm
L_a = actual length of the graticule line, mm
L_o = magnified length of the graticule line, µm, and
D = projected field diameter of the graticule, µm.

example:

If L_o = 108 µm, L_a = 2.93 mm and D = 100 µm, then d_c = 2.71 mm.
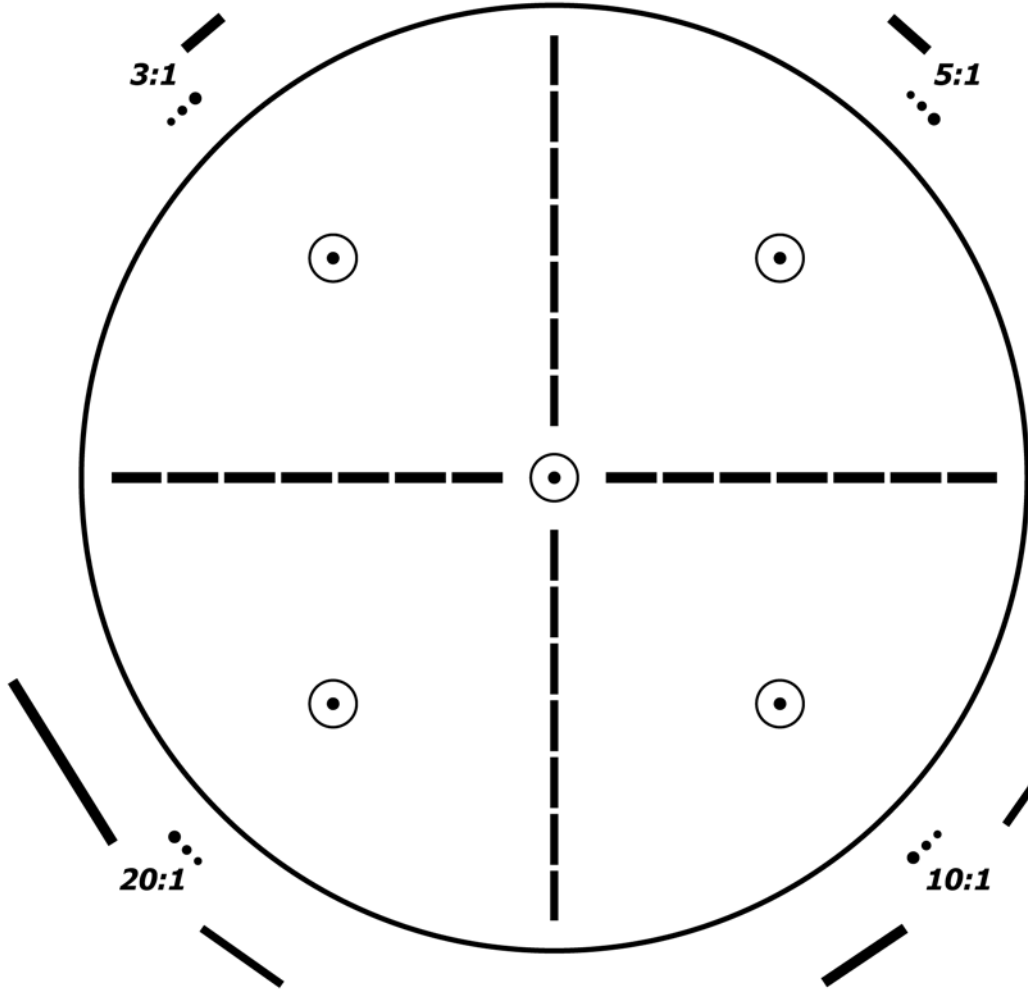
FIG. A1.1 Design of RIB Eyepiece Graticule

A1.4 Calibration of the Eyepiece Graticule

A1.4.1 Upon receipt of the graticule from the manufacturer, calibrate the field diameter, *D*, (acceptable range 100 μm ± 2 μm), using a stage micrometer. Calculate the field area (mm^2). For a field diameter of 100 μm, the field area is 0.00785 mm^2 (acceptable range 0.00754 mm^2 to 0.00817 mm^2). Adjustment of the inter-pupillary distance on some microscopes results in a change of the microscope tube length, which will change the field area represented by the graticule. For microscopes in which the tube length is not constant, each microscopist should perform an individual calibration of the field area using a stage micrometer, and also use this individually-calibrated field area in the calculation of results, in order to account for differences in inter-pupillary distances for different operators.
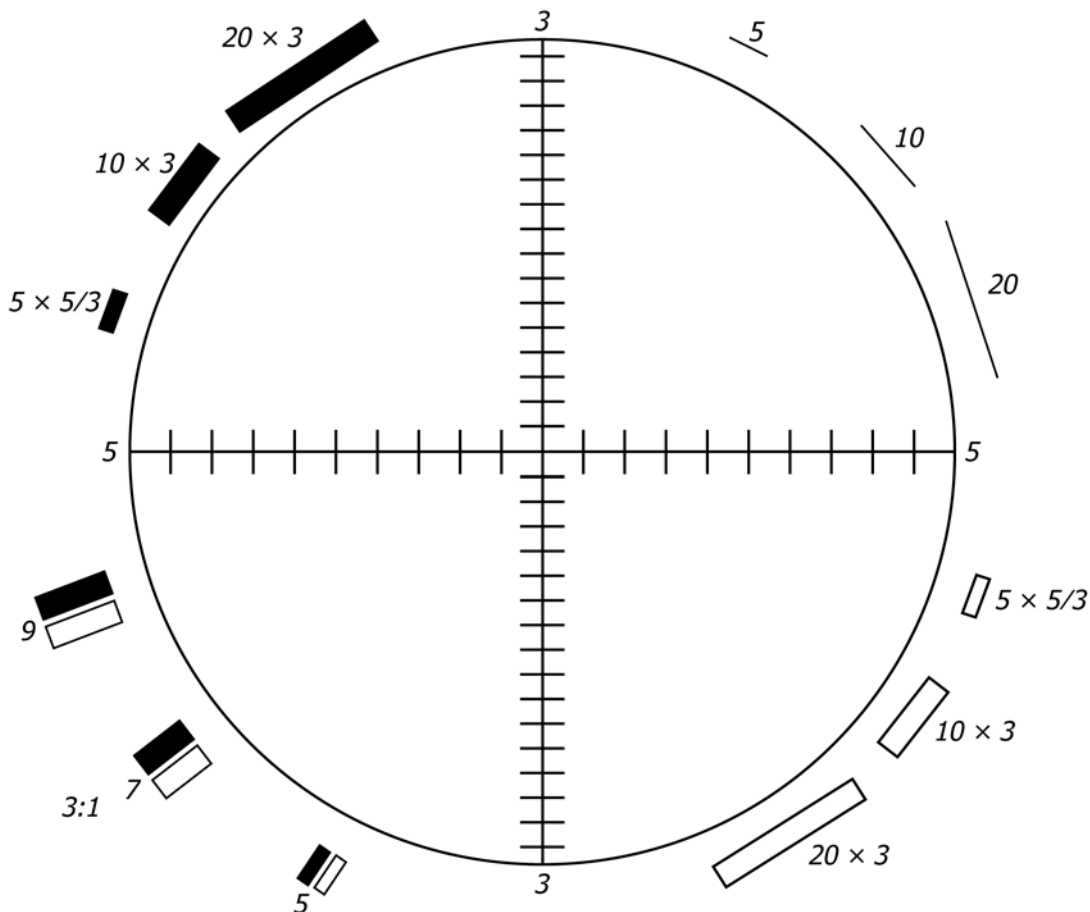
FIG. A1.2 Design of Walton-Beckett Eyepiece Graticule

A2. CALIBRATION OF THE LIMIT OF VISIBILITY USING THE HSE/NPL TEST SLIDE

A2.1 *The HSE/NPL Test Slide*—The Standard Test Slide consists of an epoxy replica of a master slide produced and certified by the National Physical Laboratory, (NPL), Teddington, United Kingdom. The design of the slide is shown in Fig. A2.1. The notations in Fig. A2.1 are not present on the actual slide; these are shown to clarify the use of the slide.

A2.1.1 The slide consists of seven blocks of grooves (approximately 20 grooves to each block) in decreasing order of visibility from Block 1 to Block 7. The seven blocks are separated by gaps approximately 20-µm wide. A set of four deep marker ridges is placed on either side of the array and two pairs of marker ridges, separated by a distance of 120 µm, intersect the two sets of four ridges at right angles. The zone of the slide to be used for testing of visibility is delineated by the rectangle bounded by the deep marker ridges.

A2.2 *Calibration Procedure*—Set up the microscope for phase contrast microscopy, and place the test slide on the microscope stage with the aluminum label strip uppermost. The circular clear area contains the testing area. Locate the test area by reference to the sets of marker ridges. The separation between the two pairs of marker ridges is just larger than the diameter of the graticule. The first two or three sets of grooves should be readily seen. Bring the first group of ridges (Block 1) into the center of the field of view. Bring the ridges into sharp focus, and then move the slide so as to position the second group of ridges in the centre of the field of view, and then focus again. Continue to move the slide to examine successive blocks of ridges of decreasing visibility. A block is defined as visible if all ridges of the block can be seen for their whole lengths between the horizontal marker ridges. It may be necessary to adjust the illumination so as to give the best results. It is unlikely that all of the blocks will be visible, even with the greatest of care and concentration. However, the majority of observers with medium to good microscopes can see the grooves of Block 5. Once the operator becomes familiar, use of the test slide on a microscope system that is correctly set up, it will become easier for the system to be checked on a regular basis. The requirements for fiber counting are that the microscope optics must resolve the grooved lines in Block 3 completely, although they may appear somewhat faint, and that the grooved lines in Block 6 and Block 7 must be invisible.
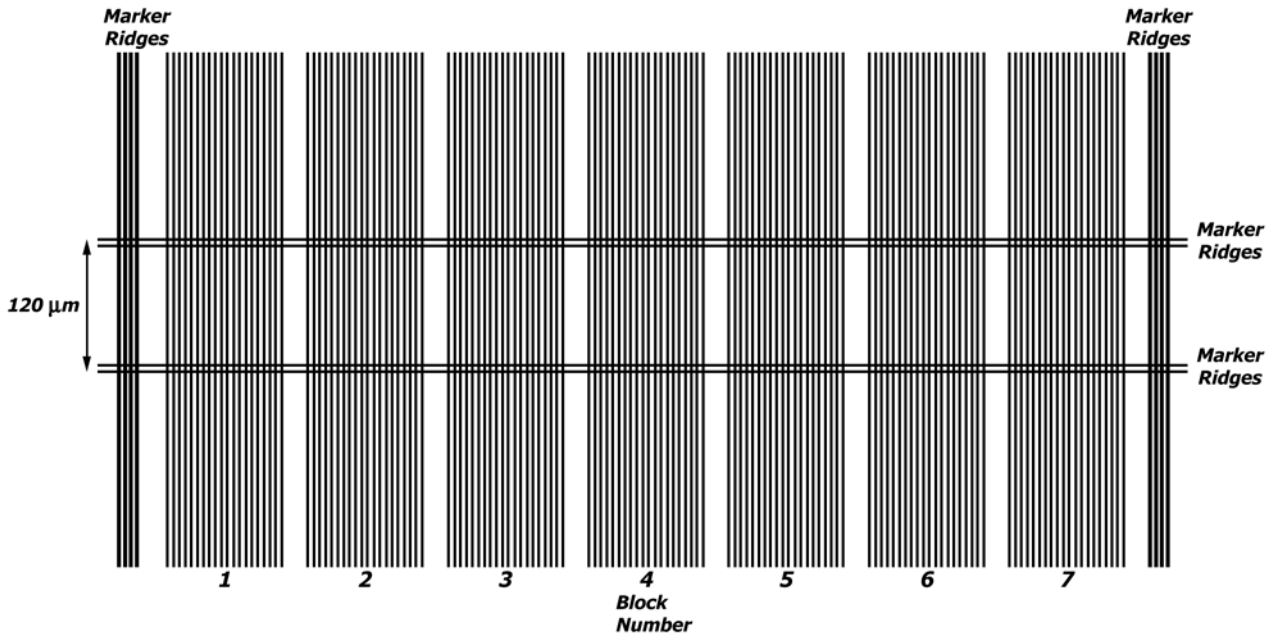
FIG. A2.1 HSE/NPL Phase Test Slide

Blocks 4 and 5 must be at least partially visible, but may vary slightly in visibility between microscopes. A microscope/microscopist combination, which fails to meet these requirements has either too low or too high a resolution and may not be used for counting fibers by this method.

A3. FIBER COUNTING CRITERIA FOR ASBESTOS ("A" RULES)

A3.1 *Counting Criteria*—Do not mix counting rules recommended by different agencies or standards. Designate all particles having the following geometric dimensions as fibers: length greater than 5 μm, (diameter smaller than 3 μm when using the rules in ISO 8672 (there is no upper limit in NIOSH 7400 or OSHA ID 160), and a minimum length to diameter ratio of 3:1.

A3.1.1 Examples to illustrate the fiber counting criteria are shown in Fig. A3.1, and these are explained in Table A3.1.

A3.1.2 Accuracy is important, and full use should be made of known dimensional standards. Estimate the length of curved fibers along the curve of the fiber (that is, the true length).

A3.1.3 Split fibers are counted as one fiber according to their geometric dimensions. The width of a split fiber is measured across the compact part of the fiber, not across the split part.

A3.1.4 Grouped fibers are counted individually where individual fibers can be distinguished. Where fibers in a bundle cannot be distinguished as individual fibers they are counted as equivalent to one fiber (unless the width of the bundle exceeds 3 μm when using the rules in ISO 8672).

A3.1.5 Fibers attached to particulate matter are counted as one fiber if the width of the particulate is less than 3 μm, otherwise they are not counted. Exclusion from counting because of any attached particle is not part of OSHA ID 160 or NIOSH 7400 A rules.

A3.1.6 Count any fiber that lies entirely within the counting area.

A3.1.7 Count as a half fiber any fiber with only one end lying within the counting area.

A3.1.8 If a single curved fiber has each end inside the counting area having crossed the area twice, each end is still counted as half a fiber.

A3.1.9 If more than one-eighth (in accordance with OSHA ID 160; or one-sixth in accordance with NIOSH 7400) of the counting field contains an agglomerate of fibers or dust, or both, reject the field and select another. Always record such occurrences.

A3.1.10 Count as many fields as are necessary to give a total fiber count of 100. A minimum of 20 fields must be counted, even if more than 100 fibers have been observed. It is not necessary to count more than 100 fields. However errors will be large when the minimum number of fibers is counted.
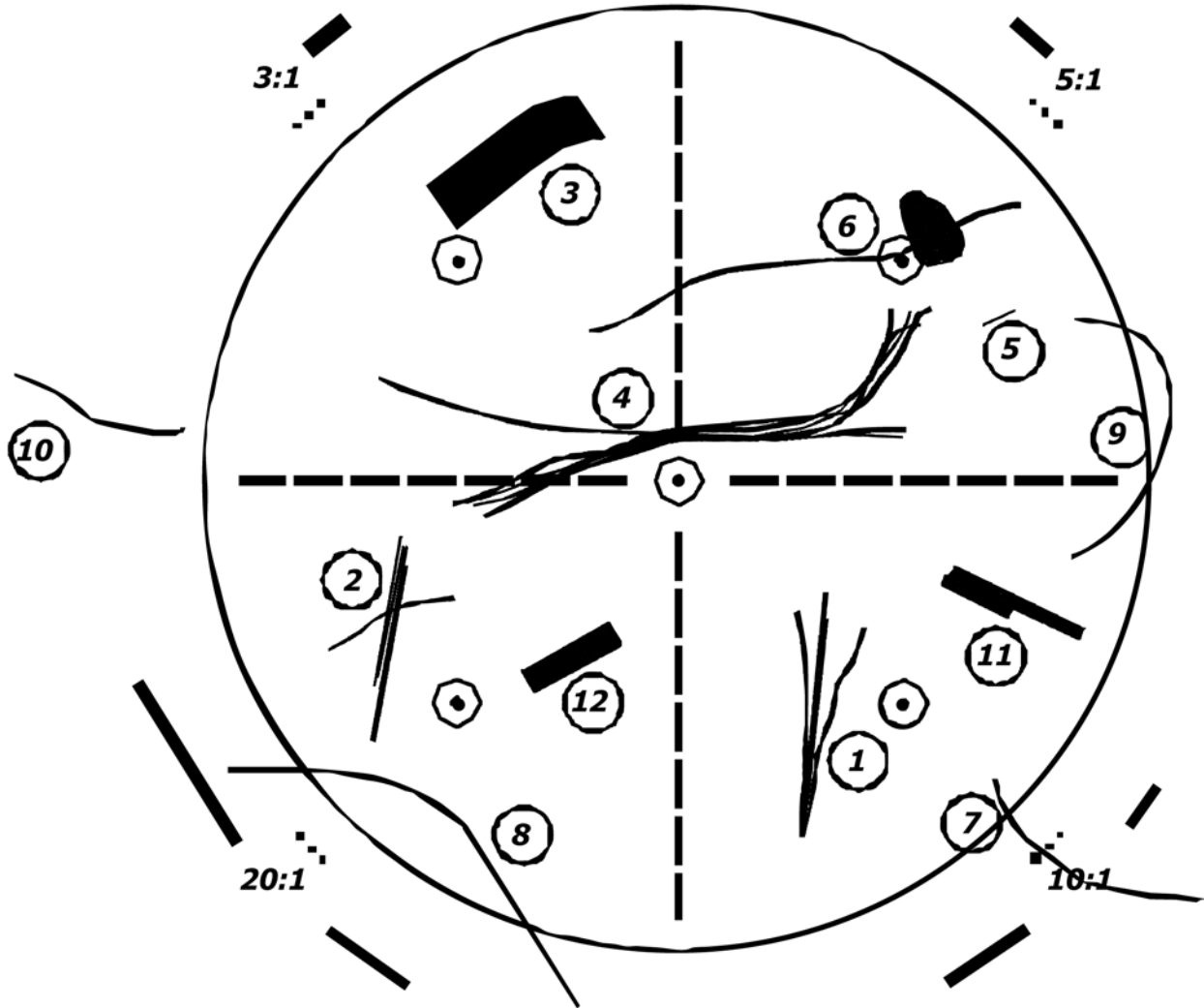
FIG. A3.1 Examples of Fibers Viewed Against a RIB Graticule.

TABLE A3.1 Examples of the use of the "A" counting criteria for the fibers shown in Fig. A3.1

Particle	Count	Reason
1	1	Optically-visible asbestos fibers are often bundles of fine fibrils. If the fibrils appear to be components of the same bundle, count the object as a single fiber.
2	2	Count intersecting fibers that individually meet the length and aspect ratio criteria (length >5 μm and minimum aspect ratio 3:1) as separate fibers.
3	1 (0)	Although the fiber is relatively large, it is counted under the NIOSH 7400 "A" rules. There is no upper limit on particle width under the "A" rules or OSHA ID 160, although there is an upper width of 3μm in the rules for ISO 8672, in which case this fiber would not be counted.
4	1	The long fibrils appear to be part of the overall bundle. Count the object as 1 fiber.
5	0	Do not count. The fiber is shorter than 5 μm.
6	1	Count a fiber partially obscured by a particle as 1 fiber. If the fiber ends emerging from the obscured area do not appear to be from the same fiber, count each as a separate fiber if the visible portion meets the length and aspect ratio criteria.
7	½	Count a fiber that crosses the ring defining the graticule area as a ½ fiber.
8	0	Do not count a fiber that has no ends within the ring defining the graticule area.
9	0 (1)	NIOSH 7400 and OSHA ID 160 do not count fibers that cross the graticule more than once. ISO 8672 counts a fiber that has both ends within the ring defining the graticule area.
10	0	Do not count a fiber that lies totally outside the ring defining the graticule area.
11	1	Count as 1 fiber.
12	1	Count as 1 fiber. Aspect ratio is higher than 3:1.

A4. DIFFERENTIAL FIBER COUNTING

A4.1 The example fibers that meet the general definition of "countable" (that is, length >5 μm, aspect ratio >3:1) shown in Fig. A3.1 will be binned as follows, in accordance with the counting rules in Section 13.13.

A4.2 *Group 1*—Those fibers that are thinner than 1.0 μm. The single fiber crossing particle 2 and particle 6 meet this definition. The individual component fibers of particle 7 also meet this definition. It has the appearance of a bundle so would be included in Group 2 even if the individual component fibers were thicker.

A4.3 *Group 2*—Those fibers that are thicker than 1.0 μm, but which show curvature or have splayed ends, or have the appearance of a bundle. Particles 1, 3, 4, and 7 are in this group. Note that particle 3 would not be counted under ISO counting rules, as being wider than 3 μm. The wider of the two fibers that make up particle 2 is also classified in Group 2 as it has the appearance of a bundle.

A4.4 *Group 3*—All other countable fibers. Particles 11 and 12 are Group 3. It might be argued that particle 3 belongs in Group 3 because of its width, but it displays some curvature, so it should be placed in Group 2.

APPENDIX

(Nonmandatory Information)

X1. IDENTIFICATION AND COUNTING OF PCM-EQUIVALENT FIBERS BY TRANSMISSION ELECTRON MICROSCOPY

X1.1. Scope

X1.1.1 This method is used to determine asbestos fibers in the optically visible range and is intended to complement the results obtained by phase contrast microscopy.

X1.1.2 The quantitative working range of this method extends from 100 fibers/mm^2 to 1300 fibers/mm^2 of filter area. For a 1000 L air sample, this corresponds to a concentration range from approximately 0.04 to 0.5 fiber/mL (or fiber/cm^3). Lower and higher ranges of fiber concentration can be measured by reducing or increasing the volume of air collected.

X1.1.3 The limit of detection for this method is one identified asbestos fiber above the 95% confidence limit for analysis of blank filters.

X1.2. Applicable documents

X1.2.1 *ASTM Standards:*
D6281 Test Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM)
X1.2.2 *Other Documents:*
ISO 10312 (**23**)[10]
NIOSH Method 7402 (Revised 1994) (**24**)[5]

X1.3. Terminology (Additional Terms Specific to This Appendix)

X1.3.1 *acicular*—the shape shown by an extremely slender crystal with cross-sectional dimensions, which are small relative to its length, that is, needle-like.

X1.3.2 *amphibole*—a group of more than 60 different silicate minerals with similar crystal structures and complex compositions that conform to the nominal formula:

$$A_{0-1}B_2C_5T_8O_{22}(OH,F,Cl)_2 \qquad (X1.1)$$
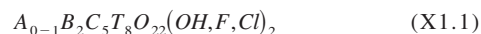
where:
A = K, Na, Ca
B = Fe^{2+}, Mn, Mg, Ca, Na
C = Al, Cr, Ti, Fe^{3+}, Mg, Fe^{2+}, Mn, and
T = Si, Al, Cr, Fe^{3+}, Ti.

In some varieties of amphibole, these elements can be partially substituted by Li, Pb, Zn, Be, Ba, or Ni. Amphiboles are characterized by a complex monoclinic or orthorhombic structure that includes a double chain of T-O tetrahedral with a T:O ratio of approximately 4:11; a variable morphology that ranges from columnar to prismatic to acicular to fibrous; and good prismatic cleavage at angles of about 56° and 124°. The cleavage may not be readily exhibited by small crystals that are bound by irregular growth and fracture surfaces (**7**).

X1.3.3 *amphibole asbestos*—amphibole in an asbestiform habit.

X1.3.4 *analytical sensitivity*—the calculated airborne asbestos fiber concentration in fibers/mL (or fibers/cm^3), equivalent

---

[10] This Appendix is based on NIOSH Method 7402 and ISO 10312. These methods have different definitions of countable fibers. The minimum diameter for NIOSH Method 7402 is 0.25 μm while that for ISO 10312 is 0.2 μm. NIOSH Method 7402 has no upper limit on fiber width, while that for ISO 10312 is 3.0 μm. The ISO definition has been used in the text, but the NIOSH definition may be substituted.

to counting of one asbestos fiber in the analysis. This method does not specify a unique analytical sensitivity.

X1.3.5 *asbestos structure*—a term applied to an individual fiber, or any connected or overlapping grouping of asbestos fibers or bundles, with or without other particles.

X1.3.6 *camera length*—the equivalent projection length between the specimen and its electron diffraction (ED) pattern, in the absence of lens action.

X1.3.7 *cleavage*—the breakage of a mineral along one of its crystallographic directions.

X1.3.8 *cluster*—a structure in which two or more fibers, or fiber bundles, are randomly oriented in a connected grouping.

X1.3.9 *d-spacing*—the distance between identical adjacent and parallel planes of atoms in a crystal.

X1.3.10 *electron diffraction (ED)*—a technique in electron microscopy by which the crystal structure of a specimen is examined.

X1.3.11 *electron scattering power*—the extent to which a thin layer of substance scatters electrons from their original directions.

X1.3.12 *energy dispersive X-ray analysis (EDXA)*— measurement of the energies and intensities of X rays by use of a solid state detector and multi-channel analyzer system.

X1.3.13 *eucentric*—the condition when the area of interest of an object is placed on a tilting axis at the intersection of the electron beam with that axis and is in the plane of focus.

X1.3.14 *fibril*—a single fiber of asbestos that cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances.

X1.3.15 *countable fiber*—an elongated particle that has parallel or stepped sides. For the purposes of this appendix, a countable fiber is defined to have an aspect ratio equal to or greater than 3:1, a length greater than 5 μm, and a width between 0.2 μm and 3.0 μm (ISO 8672), or greater than 0.2 μm (OSHA ID 160, NIOSH 7400) with no upper boundary on the width. Fibers in the same dimensional range as those measured by Phase Contrast Microscopy are also referred to as "PCM equivalent fibers."

X1.3.16 *fiber bundle*—a structure composed of parallel, smaller diameter fibers attached along their lengths. A fiber bundle may exhibit diverging fibers at one or both ends. A fiber bundle is defined as a countable fiber if its overall dimensions conform to the definition of a countable fiber

X1.3.17 *fibrous structure*—a fiber, or connected grouping of fibers, with or without other particles.

X1.3.18 *matrix*—a structure in which one or more fibers, or fiber bundles, touch, are attached to, or partially concealed by, a single particle or connected group of non fibrous particles.

X1.3.19 *Miller index*—a set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes.

X1.3.20 *PCM-equivalent fiber*—a fiber of aspect ratio greater than or equal to 3:1, longer than 5 μm, and that has a diameter between 0.2 μm and 3.0 μm (ISO 8672), or greater than 0.2 μm (OSHA ID 160, NIOSH 7400) with no upper boundary on the width.

X1.3.21 *PCM-equivalent structure*—a fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 μm, and that has a lower diameter equivalent to the least diameter that can be resolved using an optical microscope (0.2 μm, in accordance with ISO 10312, or 0.25 μm, in accordance with NIOSH Method 7402) and 3.0 μm.

X1.3.22 *primary structure*—a fibrous structure that is a separate entity in the TEM image.

X1.3.23 *replication*—a procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made.

X1.3.24 *selected area electron diffraction (SAED)*— a technique in electron microscopy in which the crystal structure of a small area of a sample is examined.

X1.3.25 *structure*—a single fiber, fiber bundle, cluster or matrix

X1.3.26 *twinning*—the occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law.

X1.3.27 *unopened fiber*—a large diameter asbestos fiber bundle that has not been separated into its constituent fibrils or fibers.

X1.3.28 *zone axis*—the line or crystallographic direction through the center of a crystal that is parallel to the intersection edges of the crystal faces defining the crystal zone.

X1.4. Summary of Method

X1.4.1 A sector of the MCE filter is chemically treated using a mixture of dimethylformamide, acetic acid and water to collapse the pore structure of the filter. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure using either dimethylformamide or acetone.

X1.4.2 The TEM specimen grids are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative fiber count on randomly-selected grid openings. In the TEM analysis, ED is used to examine the crystal structure of a fiber, and its elemental composition is determined by EDXA. Only fibers with a minimum aspect ratio of 3:1, lengths greater than 5 μm, and widths between 0.2 μm and 3.0 μm (PCM-equivalent fibers) are counted. The ratio of asbestos fibers to the total number of fibers counted is applied to the original PCM fiber count to derive the concentration of asbestos fibers in the reported PCM measurement. Alternatively, the number of PCM-equivalent asbestos fibers found on a known area of the microscope sample, together with the volume of air filtered through the sample collection filter, and the active area of the filter may be used to calculate the airborne concentration of PCM-equivalent asbestos fibers directly, without reference to an earlier PCM fiber count.

X1.5. Significance and Use

X1.5.1 The user of this practice must determine for themselves whether the practices described meet the requirements of local or national authorities regulating asbestos or other fibrous hazards.

X1.6 Interferences

X1.6.1 High levels of non fibrous dust particles may obscure fibers in the field of view and increase the limit of detection.

X1.6.2 Fragments of non asbestiform anthophyllite, tremolite and actinolite that have dimensions within the range of the fiber definition in this method will be included in the fiber counting result. If non asbestiform cleavage fragments appear to represent a significant proportion of the measurement, it is recommended that a sufficient number of fragments be measured so that the aspect ratio distribution can be determined. This distribution may provide a means of discriminating between asbestos and non asbestiform cleavage fragments.

X1.6.3 *Interferences*—Other amphibole particles that have aspect ratios greater than 3:1 and elemental compositions similar to the asbestos minerals may interfere in the TEM analysis. Some non-amphibole minerals may give ED patterns similar to those from amphiboles.

X1.7. Apparatus

X1.7.1 *Specimen Preparation Laboratory*—Asbestos, particularly chrysotile, is present in varying quantities in many laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibers, which may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that during preparation, contamination of TEM specimens by any extraneous asbestos fibers is minimized. All specimen preparation steps shall therefore be performed in an environment where the potential for contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result lower than 1 fiber/mm^2 for PCM-equivalent fibers. A minimum facility considered suitable for preparation of TEM specimens is a positive pressure, laminar flow hood. However, it has been established that work practices during specimen preparation appear to be more important than the type of clean handling facilities in use. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE X1.1—It is recommended that activities involving manipulation of bulk asbestos samples not be performed in the same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

X1.7.2 *Transmission Electron Microscope:*

X1.7.2.1 A TEM operating at an accelerating potential of 80-120 kV, with a resolution better than 1.0 nm at 100 000× magnification, and a magnification range of approximately 300 to 100 000 shall be used. The ability to obtain a direct screen magnification of about 100 000 is necessary for inspection of fiber morphology; this magnification may be obtained by supplementary optical enlargement of the screen image with a binocular if it cannot be obtained directly. The viewing screen shall be calibrated (such as shown in Fig. X1.1) with concentric circles and a millimetre scale such that the lengths and widths of fiber images down to 1 mm width can be measured in increments of 1 mm.

X1.7.2.2 For Bragg angles less than 0.01 radians the TEM shall be capable of performing ED from an area of 0.6 µm^2 or less, selected from an in focus image at a screen magnification of 20 000. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If SAED is used, the performance of an individual instrument may normally be calculated using the following relationship:

$$A = 0.7854 \left(\frac{D}{M} + 2000 C_s \Theta^3 \right)^2 \qquad (X1.2)$$

where:

A = the effective SAED area in µm^2
D = the diameter of the SAED aperture in µm
M = the magnification of the objective lens
C_s = the spherical aberration coefficient of the objective lens in mm
2 = maximum required Bragg angle in radians

X1.7.2.3 It is not possible to reduce the effective SAED area indefinitely by the use of progressively smaller SAED apertures, because there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

X1.7.2.4 If zone axis ED analyses are to be performed, the TEM shall incorporate a goniometer stage that permits the TEM specimen to be either:

X1.7.2.4.1 Rotated through 360°, combined with tilting through at least +30° to −30° about an axis in the plane of the specimen; or,

X1.7.2.4.2 Tilted through at least +30° to −30° about two perpendicular axes in the plane of the specimen.

X1.7.2.5 The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fiber, the goniometer shall be of a type that permits tilting of the specimen and acquisition of EDXA spectra without change of specimen holder.

X1.7.2.6 The TEM shall have an illumination and condenser lens system capable of forming an electron probe smaller than 250 nm in diameter.

X1.7.3 *Energy Dispersive X-ray (EDXA) Analyzer:*

X1.7.3.1 The TEM shall be equipped with an EDXA analyzer capable of achieving a resolution better than 180 eV (FWHM) on the Mn Kα peak. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of geometrical factors, the required performance of the combination of the TEM and X-ray analyzer is specified in terms of the measured X-ray intensity obtained from a fiber of small diameter, using a known electron beam diameter. Solid state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyzer shall yield, under
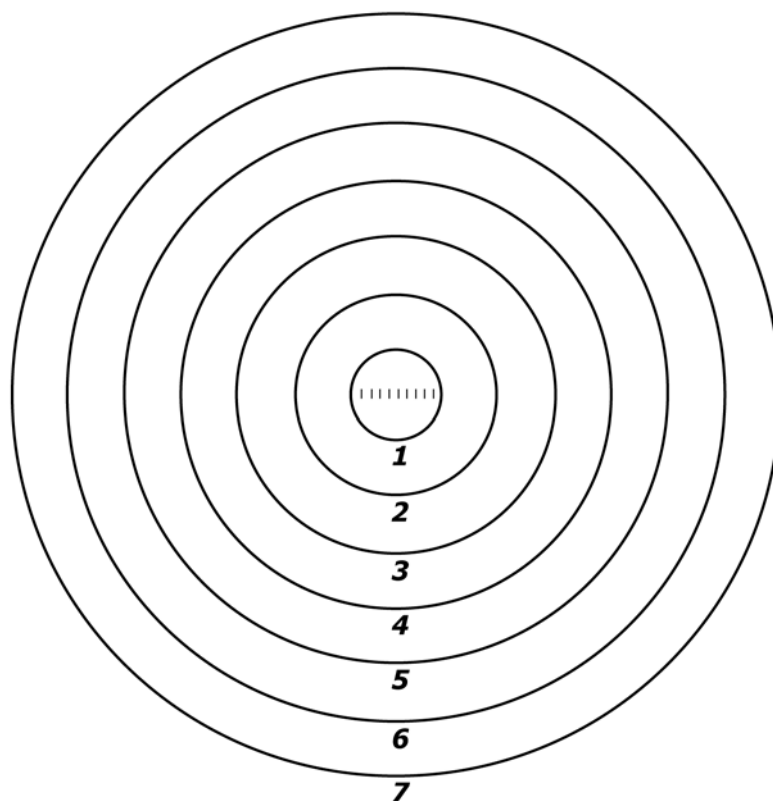
FIG. X1.1 Example of Calibration Markings on TEM Viewing Screen

routine analytical conditions, a background-subtracted Na Kα integrated peak count rate of more than 1 count per second (cps) from a fiber of UICC crocidolite 50 nm in diameter or smaller when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1.0.

X1.7.3.2 The EDXA unit shall provide the means for subtraction of the background, identification of elemental peaks, and calculation of background subtracted peak areas.

X1.7.4 *Vacuum Coating Unit*—A vacuum coating unit capable of producing a vacuum better than 0.013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample holder is required that will allow a glass microscope slide to be continuously rotated during the coating procedure.

NOTE X1.2—A mechanism that allows the rotating slide also to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

X1.7.5 *Sputter Coater*—A sputter coater with a gold target may be used for deposition of gold on to TEM specimens as an internal calibration of ED patterns. Other calibration materials are acceptable. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

X1.7.6 *Solvent Washer (Jaffe Washer)*—The purpose of the Jaffe washer is to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibers and other particles from the filter surface. One design of a washer that has been found satisfactory for various solvents and filter media is shown in Fig. X1.2, and dimethylformamide or acetone have been found satisfactory for dissolving MCE filters. The higher evaporation rate of acetone requires that a reservoir of 10-50 mL of solvent be used, and it may be necessary to replenish the solvent during the procedure. Dimethylformamide has a much lower vapor pressure, and a smaller volume of solvent may be used without the need to replenish it during the dissolution. It is recommended that all washers be used in a fume hood, and when specimens are not being inserted or removed, during the solvent dissolution the Petri dish lid shall be in place. Clean the washer before it is used for each batch of specimens.

X1.7.7 *Slide Warmer or Oven*—Use either a slide warmer or an oven for heating slides during the preparation of TEM specimens from cellulose ester filters. It is required to maintain a temperature of 65–70°C.

X1.7.8 *Carbon Grating Replicas*—A carbon grating replica with about 2000 parallel lines per mm shall be used to calibrate the magnification of the TEM at 5000 and 20 000. For calibration at a magnification of approximately 1000, for measurement of individual grid opening dimensions in the TEM, a carbon grating replica of approximately 600 lines/mm is recommended.
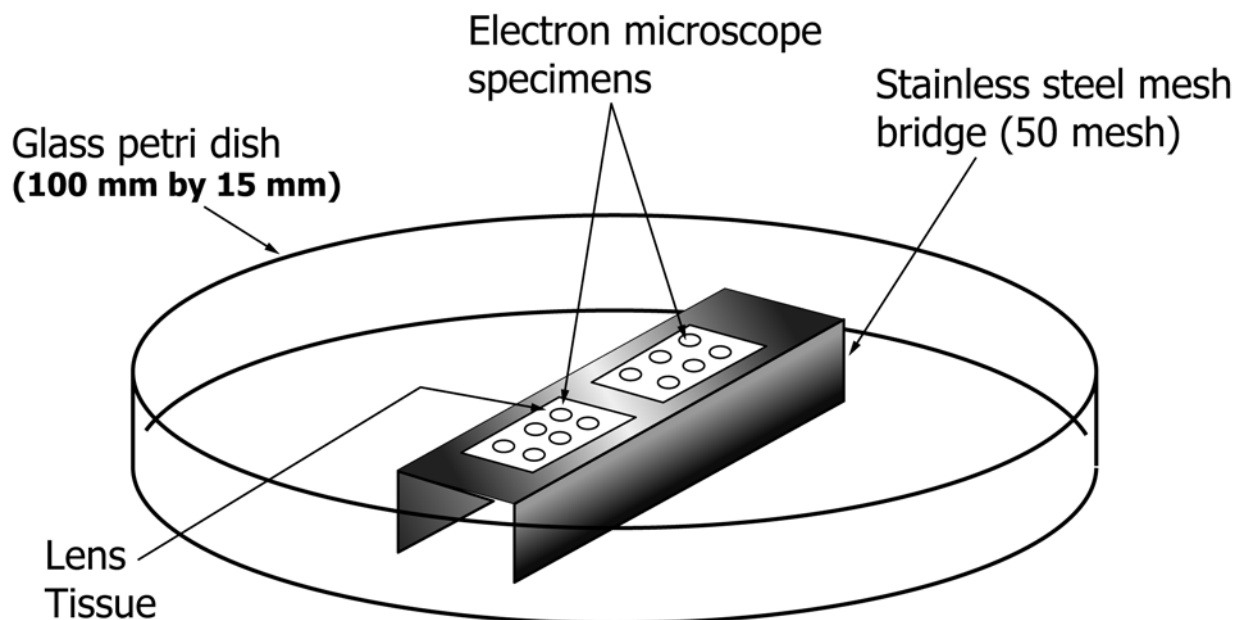
FIG. X1.2 Example of Design of Solvent Washer (Jaffe Washer). (Solvent is added until the meniscus contacts the underside of the stainless steel mesh.)

X1.7.9 *Calibration Grids for EDXA*—TEM specimen grids prepared from dispersions of calibration minerals are required for calibration of the EDXA system. Examples of suitable calibration minerals are riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. Prepare the mineral used for calibration of the EDXA system for sodium using a gold TEM grid.

X1.7.10 *Carbon Rod Sharpener*—The use of necked carbon rods, or equivalent, allows the carbon to be evaporated on to the filters with a minimum of heating.

X1.7.11 *Copper Electron Microscope Grids*—Two hundred mesh TEM grids are recommended. Use grids that have grid openings of uniform size such that the standard deviation of the mean of ten openings selected from ten grids is less than 5 %. Optionally, or if the 5 % standard deviation criterion cannot be demonstrated, measure the dimensions of each grid opening examined in the TEM at a calibrated magnification. To facilitate the re-location of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended. Commercially available grids with a certified calibration may be used.

X1.7.12 *Gold Electron Microscope Grids*—Two hundred mesh gold TEM grids are recommended to mount TEM specimens when sodium measurements are required in the fiber identification procedure. Use grids that have grid openings of uniform size such that the standard deviation of the mean of ten openings selected from ten grids is less than 5 %. Optionally, or if the 5 % standard deviation criterion cannot be demonstrated, measure the dimensions of each grid opening examined in the TEM at a calibrated magnification. To facilitate the re-location of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

X1.7.13 *Carbon Rod Electrodes*—Spectrochemically pure carbon rods, for use in the vacuum evaporator during carbon coating of filters.

X1.7.14 *Routine Electron Microscopy Tools and Supplies*—Fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, lens tissue, gold wire, tungsten filaments and other routine supplies are required.

X1.7.15 *Reference Asbestos Samples*—Asbestos samples, for preparation of reference TEM specimens of the primary asbestos minerals. Either the UICC set of minerals or SRM 1866 and SRM 1867 are suitable for this purpose.

X1.8. Reagents

X1.8.1 *Fiber Free Water.*

X1.8.2 *Dimethylformamide*, analytical grade .

X1.8.3 *Glacial Acetic Acid*, analytical grade.

X1.8.4 *Acetone*, analytical grade. (**Warning**—Use the reagents in accordance with the appropriate health and safety regulations.)

X1.9. Preparation of TEM specimens

X1.9.1 *Selection of Area of Filter for Preparation*—Using clean tweezers, remove the membrane filter from the cassette, and place it on a cleaned microscope slide. Using a clean, curved scalpel blade, cut out a 90° sector of the filter.

X1.9.2 *Preparation of Solution for Collapsing Membrane Filters*—Mix 35 mL of dimethyl formamide, 15 mL of glacial acetic acid with 50 mL of freshly-distilled and filtered water. Store this mixture in a clean bottle. The mixture is stable and suitable for use for up to three months after preparation.

X1.9.3 *Filter Collapsing Procedure*—Using a micropipet with a disposable tip, place 15-25 µL/cm^2 of the collapsing

solution on a cleaned microscope slide, and using the end of the pipet tip spread the liquid over the area to be occupied by the filter portion. Place the filter portion, with the active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20 degrees so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter portion may be placed on one slide. Place the slide either on a thermostatically controlled slide warmer at a temperature of 65-70°C, or in an oven at this temperature, for 10 minutes. The filter collapses slowly to about 15 % of its original thickness. The procedure leaves a thin, transparent polymer film, with particles and fibers embedded in the upper surface. Alternative solvent procedures for collapsing the filter that can be shown to produce the same result may also be used.

NOTE X1.3—Since the intention of the analysis is to determine the proportion of the total number of fibers counted in the PCM measurement that are the regulated fibers under consideration, it is important to include organic fibers such as cellulose in the TEM fiber count. Accordingly, the collapsed membrane filter shall not be plasma etched If the sample is ashed to remove organic fibers a much faster count of 100 asbestos fibers can be achieved, from which a concentration can be calculated directly, rather than by adjusting a previous PCM count. However, it should be noted that these two methods are unlikely to give identical results, and the second method is not favored by all regulatory agencies.

X1.9.4 *Carbon Coating of the Membrane Filter*—Place the glass slide holding the filter portions on the rotation tilting device, approximately 10-12 cm from the evaporation source, and evacuate the evaporator chamber to a vacuum better than 0.013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by a few seconds to allow the electrodes to cool. The thickness of carbon required is dependent on the size of the particles on the filter, and approximately 30-50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image that is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

X1.9.5 *Preparation of the Jaffe Washer*—Place a stainless steel bridge in the washer, and place three copper specimen grids on the stainless steel mesh for each filter sample being prepared (use of the lens tissue is optional). Fill the washer with dimethylformamide or acetone to a level where the meniscus contacts the underside of the mesh.

X1.9.6 *Placing of TEM Specimens into the Jaffe Washer*—Using a curved scalpel blade, cut three 3-mm square pieces of carbon coated membrane filter from the carbon coated filter sector. Select three squares to represent the centre and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on to one of the TEM specimen grids on the stainless steel mesh in the Jaffe washer. Several specimens may be prepared in the same Jaffe washer. Cover the Jaffe washer with the lid. Specimens are normally cleared of

filter polymer after approximately 1 hour if dimethylformamide is used as the solvent, and approximately 4 hours if acetone is used.

X1.10 Measurement

X1.10.1 *Criteria for Acceptable TEM Specimen Grids*—Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Reject the grids if:

X1.10.1.1 The carbon replica covers less than approximately 75 % of the area of the TEM specimen grids.

X1.10.1.2 The TEM specimen has not been cleared of filter medium by the filter dissolution step. If the TEM specimen exhibits areas of undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional solvent washing shall be carried out, or new specimens shall be prepared from the membrane filter.

X1.10.1.3 More than approximately 25 % of the grid openings have broken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the fiber count. Prepare new specimens from the membrane filter, using a heavier carbon coating, or using carbon coated specimen grids.

X1.10.1.4 Specimens have more than 25 % of the area covered by non-fibrous particulate.

X1.10.2 *Calibration:*

X1.10.2.1 *Calibration of the TEM Magnifications*—Using the diffraction grating replicas, calibrate the magnification of the TEM at the magnification settings closest to 1000, 5000 and 20 000. After inserting the replica, ensure that the goniometer tilt angle is set to zero, and also that the specimen is eucentric. Measure the distance on the fluorescent screen occupied by a specific number of rulings of the grating. Calculate the magnification using this measurement and the data for the specific diffraction grating. Alternatively, a TEM micrograph may be taken of the diffraction grating, and the magnification ratio for the camera and the fluorescent screen (which is a constant for each model of TEM) can be used to calculate the screen magnification from measurements made on the recorded negative.

X1.10.2.2 *Determination of the Grid Opening Dimensions*—Measure 20 grid openings at random on a 200 mesh copper grid selected from the batch of grids in use by placing a grid on a glass slide and examining it using the optical microscope. Use the Walton-Beckett graticule or other appropriate eyepiece graticule to measure the grid opening dimensions. Calculate an average grid opening dimension from the data and use this to calculate the average grid opening area. Alternatively, the dimensions of one grid opening one each grid examined may be measured at a calibrated magnification close to 1000 in the TEM. Calculate the average grid opening area for the three sets of measurements, and use this area in the calculation of results for the sample.

X1.10.2.3 Prepare TEM specimen grids from each of the reference asbestos fiber samples in X1.7.15, and also from each of the calibration minerals referenced in X1.7.9. Acquire and record EDXA spectra from typical fibers or particles, and

maintain a current library of these spectra for use in identification of fibers. The performance and resolution of EDXA detectors degrade over time, and it is important to check the performance of the detector regularly using the calibration minerals. ED patterns from the asbestos varieties should also be examined and recorded. This method does not require quantitative interpretation of ED patterns. For chrysotile, observation of the morphology and qualitative observation of an ED pattern is usually sufficient to establish its identity.

X1.10.3 *Examination of TEM Specimens*—Use a magnification of approximately 5000 (ISO 10312) or 1000 (NIOSH Method 7402) for counting of fibers and bundles that have a minimum aspect ratio of 3:1, are longer than 5 µm, and are within the diameter range 0.2 µm to 3.0 µm. For each fiber, record the length and width in millimetres, micrometres, or nanometres as observed on the fluorescent screen of the TEM, and the identification of the fiber. In order to measure the width of a fiber accurately in the region of 0.2 µm, it is necessary to increase the magnification to approximately 20 000. In practice, it is most convenient to measure lengths at 5000 magnification and widths at 20 000 magnification. Continue the count until completion of the grid opening on which the 100th fiber has been recorded, or until a specimen area of 0.785 mm^2, corresponding to the area of filter examined in the PCM fiber counting method, has been examined. The number of grid openings that is equivalent to this area may vary depending on the source of the grid. It may be possible to measure a smaller area for comparison purposes. If the intention is to measure the concentration of PCM-equivalent fibers independently of the PCM measurement, only those structures that are identified as, or are suspected to be, the regulated species should be reported in the TEM examination. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest. The grid openings examined shall be divided approximately equally between the three specimen grids.

NOTE X1.4—PCM-equivalent fibers within this dimensional range do not necessarily incorporate fibers or bundles longer than 5 µm.

X1.10.4 *Fiber Counting Criteria*—Distribute the grid openings to be examined approximately equally among the three grid preparations. Count only grid openings that have the carbon film intact. At 5000 magnification, begin counting at one end of the grid and systematically traverse the grid by rows, reversing direction at row ends. Select the number of grid openings per traverse based on the fiber loading observed, so that the data can be appropriately divided between the three grids. As a guideline, count all fibers that would have been counted in the PCM examination. Count fibers using the following rules:

X1.10.4.1 Count all particles meeting the definition of a fiber (longer than 5 µm, width greater than 0.2 µm, and a minimum aspect ratio of 3:1). Use a higher magnification (approximately 20 000) to determine the widths of any fibers close to the minimum width criterion. Acquire an EDXA spectrum to analyze each suspected amphibole fiber. Examine the SAED pattern from each suspected chrysotile fiber bundle, and/or acquire an EDXA spectrum. Particles that are of questionable morphology should be analyzed by SAED and EDXA to aid in identification.

X1.10.4.2 Count fibers that are partially obscured by the grid bars as half fibers, but only if more than 2.5 µm of the fiber length is visible. Otherwise, do not count.

X1.10.4.3 Measure the length and width of each fiber as it is counted, and record them on a suitable form. For fibers that extend beyond the field of view, move the fiber and superimpose it on the scale until its entire length has been measured.

X1.10.4.4 After the dimensions of a fiber have been measured, return to the lower magnification of 5000 and continue the traverse of the grid opening to the next fiber.

X1.10.5 *Identification of Fibers*—Since the results from this analysis are intended to complement the results of a PCM fiber count made in an occupational environment, it is unlikely that the materials in use will be completely unknown. Accordingly, comparison of EDXA spectra with those obtained from the reference materials specified in X1.7.15 will generally provide sufficient information to establish the asbestos variety, or to specify that the composition of the fiber is not consistent with any of the common asbestos varieties. Qualitative observation of the ED pattern does not provide positive confirmation that the fiber is an amphibole, but the pattern is often useful as an indication. Countable chrysotile fibers will always be fiber bundles, and observation of the morphology combined with a visual examination of the ED pattern is sufficient identification, if chrysotile is known to be in use. If the variety of fiber in use is not one of the common asbestos varieties, a bulk sample of the material should be obtained and TEM specimens should be prepared from it, so that reference spectra for the particular material can be obtained. A full discussion of fiber identification by ED and EDXA can be found in either Test Method D6281 or ISO 10312 (**23**).

X1.10.6 *Data Recording*—On a suitable form, record the identification of the sample, the air volume collected, the grid opening dimension measurements, the TEM magnifications used, and the name of the analyst. For each grid opening examined, record the grid opening location number or letter. For each fiber longer than 5 µm, with a width greater than 0.2 µm, and a minimum aspect ratio of 3:1, record the location number or letter of the grid opening on which it was detected, its length and width (either in mm on the screen, or directly in µm), and the identification assigned to the fiber.

X1.11. Calculations

X1.11.1 Calculate the ratio of the number of asbestos fibers to the total number of fibers counted. Multiply the PCM fiber concentration by this ratio to obtain the concentration of asbestos fibers in the PCM count. The result shall be specified as "PCM-equivalent asbestos fibers." Optionally, the separate contribution of each asbestos species to the PCM measurement may also be calculated in a similar manner. The ratio may be applied to other filters for which the TEM measurement is representative only if it has been previously demonstrated that the dust cloud in the area is homogeneous with respect to the relative amounts of asbestos and non-asbestos fibers.

X1.11.2 The concentration of PCM equivalent asbestos fibers for each of the asbestos species may also be calculated independently of the PCM measurement. Use the following formula:

$$C = \frac{(A_f N)}{k A_g V_s 1000} \qquad (X1.3)$$

where:

C	=	Fiber concentration in fibers/mL (or fibers/cm^3)
A_f	=	Active area of filter in mm^2
N	=	Number of fibers counted
k	=	Number of grid openings examined
A_g	=	Area of TEM specimen grid opening in mm^2
V_s	=	Volume of air sampled in litres

X1.12 Quality Assurance

X1.12.1 One sample in every ten should be re-examined by a different microscopist. Optionally, the re-examination may be a verified fiber count (**25**). For fibers within the dimensional range of this method, false negatives and false positives should be related only to the interpretation of complex arrangements of fibers and bundles. For fibers within the dimensional range of this method, errors in fiber identification and errors in detection of fibers should be minimal.

X1.12.2 For asbestos fibers within the dimensional range of this method, backgrounds observed on field blanks should be negligible. Analyze field blanks to establish that the background of asbestos fibers is insignificant compared with the asbestos concentration reported. For fibers within the dimensional range of this method, field blank values for asbestos should always be below the maximum value specified for unused filters (less than 1 fiber/mm^2). If asbestos concentrations higher than 1 fiber/mm^2 in the dimensional range of this method are experienced on field blanks, it indicates that control over asbestos contamination has been lost, and the analytical procedures should be critically examined prior to continuing with any further analyses.

X1.13. Precision and Bias

The TEM method, using the direct count of asbestos fibers, has been shown to have a precision of 0.275 (S_r) in an evaluation of a mixture of amosite and wollastonite fibers. The estimate of the asbestos fraction, however, had a precision of 0.11 (S_r). When the calculated fraction was applied to the PCM count, the overall precision of the combined analysis was 0.20 (**26**). There is no independent method for determination of bias.

REFERENCES

(1) International Organization for Standardization, ISO 8672.

(2) National Institute of Occupational Safety and Health, "Asbestos and other Fibers by PCM.". NIOSH 7400, Issue 2, *NIOSH Manual of Analytical Methods*, 4th edition, DHHS (NIOSH) Publication 94-113 (16 August, 1994).

(3) Occupational Safety and Health Administration, OSHA method ID 160, Parts 29 CFR Parts 1910, 1915, and 1926. Occupational Exposure to Asbestos; Final Rule, Department of Labor, Occupational Safety and Health Administration, Federal Register, August 10, 1994.

(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, 505-516 (1982).

(5) Campbell, W.J., Blake, R.L., Brown, L.L., Cather, E.E., and Sjoberg, J.J., "Selected Silicate Minerals and their Asbestiform Varieties", *Information Circular 8751*, United States Bureau of Mines, 1977 .

(6) Wylie, A.G. and Huggins, C.W. "Characteristics of a potassium winchite asbestos from the Allamoore talc district, Texas". *Canadian Mineralogist*, 19, 1980, 101-107.

(7) Leake, B.E. et al., "Nomenclature of the Amphiboles: Report of the Subcommittee on Amphiboles of the International Mineralogical Association Commission on New Minerals and Mineral Names." *Can. Mineral.* 35, 1997, 219-246.

(8) Holmes, S., "Measurement of Airborne Asbestos Fiber by the Membrane Filter Method," Technical Note No. 4, Asbestos Research Council, Box 40, Rochdale, Lancs, England, 1971.

(9) Asbestos International Association, AIA Health and Safety Recommended Technical Method #1 (RTM1). "Airborne Asbestos Fiber Concentrations at Workplaces by Light Microscopy", (Membrane Filter Method), London (1979).

(10) 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 Fibres by the Membrane Filter Method," *Ann. Occup. Hyg.*, 25, 309-316 (1982).

(11) U.S. Code of Federal Regulations, 40 CFR Part 763

(12) Occupational Safety and Health Administration, Appendix A, Parts 29 CFR Parts 1910, 1915, and 1926. Occupational Exposure to Asbestos; Final Rule, Department of Labor, Occupational Safety and Health Administration, Federal Register, August 10, 1994

(13) LeGuen, J.M. M and S. Galvin, "Clearing and Mounting Techniques for the Evaluation of Asbestos Fibers by the Membrane Filter Method". *Ann Occup. Hyg.*, Vol.24, No.3, pp 273-280 (1981)

(14) Lee, R. J. "Letter to Mr. Tom Hall, OSHA", submitted as part of OSHA rulemaking, Docket H-033D, December 13, 1990.

(15) 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, 1986, 259-266.

(16) Walton, W. H. "The Nature, Hazards, and Assessment of Occupational Exposure to Airborne Asbestos Dust: A Review," *Ann. Occup. Hyg.*, 25, 1982, 115-247.

(17) 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), 1986, 465-474.

(18) Ogden, T. L. "The Reproducibility of Fiber Counts," Health and Safety Executive Research Paper 18 (1982).

(19) Asbestos Information Association, "A Study of the Empirical Precision of Airborne Asbestos Concentration Measurements in the Workplace by the Membrane Filter Method", Air Monitoring Committee Report, Arlington, VA (June, 1983).

(20) Harper, M., Lee, E.N., Slaven, J.E., Bartley, D.L., An Inter-Laboratory Study to Determine the Effectiveness of Procedures for Discriminating Amphibole Asbestos Fibers from Amphibole Cleavage Fragments in Fiber Counting by Phase-Contrast Microscopy. *Annals of Occupational Hygiene*, Vol. 56, pp. 645-659 (2012)

(21) Cowie, A.J. and Crawford, N.P. "A Comparison of the Effects of Different Counting Rules and Aspect Ratios on the Level and Reproducibility of Asbestos Fibre Counts. Part I: Effects on Level." Report No. TM/82/23 (EUR P.77), Institute of Occupational Medicine, Roxburgh Place, Edinburgh EH8 9SU, December 1982 .

(22) Crawford, N.P., Thorpe, H.L. and Alexander, W. "A Comparison of the Effects of Different Counting Rules and Aspect Ratios on the Level and Reproducibility of Asbestos Fibre Counts. Part II: Effects

on Reproducibility." Report No. TM/82/24 (EUR P.77), Institute of Occupational Medicine, Roxburgh Place, Edinburgh EH8 9SU, December 1982.

(23) International Organization for Standardization, ISO 10312.

(24) National Institute of Occupational Safety and Health, "Asbestos by TEM". NIOSH Method 7402, Issue 2, *NIOSH Manual of Analytical Methods*, 4th edition, DHHS (NIOSH) Publication 94 113 (16 August, 1994).

(25) Steel, E. B. and J. A. Small. "Accuracy of Transmission Electron Microcopy for the Analysis of Asbestos in Ambient Environments," *Anal. Chem.*, 57, 209-213 (1985).

(26) 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).