Designation: D7200 – 12

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

This standard is issued under the fixed designation D7200; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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.

[1] This practice is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.07 on Sampling and Analysis of Asbestos.

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[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.

1.10 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

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 *Definitions*—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.

[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.

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 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

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

belong to a member or members of the group of fibers regulated by that standard.

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.

[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.

9.3 *Lacquer or Nail Polish.*

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 required. 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).

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

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 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 carefully 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 modified 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:

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

PHASE CONTRAST MICROSCOPY FIBER COUNT
ASTM D7200

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										
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1										
2										
3										
1										
2										
3										
1										
2										
3										
1										
2										
3										

	Rejected Fields
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	

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

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

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)

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:

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

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:

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

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:

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

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

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