Designation: D6281 – 15

Standard Test Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM)[1]

This standard is issued under the fixed designation D6281; 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 test method[2] is an analytical procedure using transmission electron microscopy (TEM) for the determination of the concentration of asbestos structures in ambient atmospheres and includes measurement of the dimension of structures and of the asbestos fibers found in the structures from which aspect ratios are calculated.

1.1.1 This test method allows determination of the type(s) of asbestos fibers present.

1.1.2 This test method cannot always discriminate between individual fibers of the asbestos and non-asbestos analogues of the same amphibole mineral.

1.2 This test method is suitable for determination of asbestos in both ambient (outdoor) and building atmospheres.

1.2.1 This test method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn and for blank filters.

1.3 The upper range of concentrations that can be determined by this test method is 7000 s/mm^2. The air concentration represented by this value is a function of the volume of air sampled.

1.3.1 There is no lower limit to the dimensions of asbestos fibers that can be detected. In practice, microscopists vary in their ability to detect very small asbestos fibers. Therefore, a minimum length of 0.5 µm has been defined as the shortest fiber to be incorporated in the reported results.

1.4 The direct analytical method cannot be used if the general particulate matter loading of the sample collection filter as analyzed exceeds approximately 10 % coverage of the collection filter by particulate matter.

1.5 *This standard does not purport to address all of the safety concerns, if any, 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.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1193 Specification for Reagent Water

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

D1357 Practice for Planning the Sampling of the Ambient Atmosphere

D4483 Practice for Evaluating Precision for Test Method Standards in the Rubber and Carbon Black Manufacturing Industries

D6620 Practice for Asbestos Detection Limit Based on Counts

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

2.2 *ISO Standard:*[4]

ISO 10312 Ambient air - Determination of asbestos fibres - Direct-transfer transmission electron microscopy method

3. Terminology

3.1 For definitions of general terms used in this test method, refer to Terminology D1356 (see 2.1).

3.2 *Definitions of Terms Specific to This Standard:*

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

[1] This test method 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 was adapted from International Standard ISO 10312 "Air quality—Determination of asbestos fibres—Direct transfer transmission electron microscopy method."

[3] 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.

[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

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

$$A_{0-1}B_2C_5T_8O_{22}(OH,F,Cl)_2 \quad (1)$$

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 tetrahedra 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 (**1**).[5]

3.2.3 *amphibole asbestos*—amphibole in an asbestiform habit.

3.2.4 *analytical sensitivity*—the calculated airborne asbestos structure concentration in asbestos structures/L, equivalent to the counting of one asbestos structure in the analysis.

3.2.5 *asbestiform*—a specific type of fibrous habit in which the fibers are separable into thinner fibers and ultimately into fibrils. This habit accounts for greater flexibility and higher tensile strength than other habits of the same mineral.

3.2.6 *asbestos*—a collective term that describes a group of naturally occurring, inorganic, highly-fibrous, silicate minerals that are easily separated into long, thin, flexible, strong fibers when crushed or processed.

3.2.6.1 *Discussion*—Included in the definition are the asbestiform varieties of serpentine (chrysotile); riebeckite (crocidolite); grunerite (grunerite asbestos [Amosite]); anthophyllite (anthophyllite asbestos); tremolite (tremolite asbestos); and actinolite (actinolite asbestos). The amphibole mineral compositions are defined according to the nomenclature of the International Mineralogical Association.

Asbestos	Chemical Abstracts Service Registry No.[6]
Chrysotile	12001-29-5
Crocidolite	12001-28-4
Grunerite Asbestos [Amosite]	12172-73-5
Anthophyllite Asbestos	77536-67-5
Tremolite Asbestos	77536-68-6
Actinolite Asbestos	77536-66-4

3.2.7 *asbestos structure*—a term applied to isolated fibers or to any connected or overlapping grouping of asbestos fibers or bundles, with or without other nonasbestos particles.

3.2.8 *aspect ratio*—the ratio of length to width of a particle.

3.2.9 *blank*—a structure count made on TEM specimens prepared from an unused filter to determine the background measurement.

3.2.10 *camera length*—the equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action.

3.2.11 *chrysotile*—a group of fibrous minerals of the serpentine group that have the nominal composition $Mg_3Si_2O_5(OH)_4$ and have the crystal structure of either clinochrysotile, orthochrysotile, or parachrysotile. Most natural chrysotile deviates little from this nominal composition. Chrysotile may be partially dehydrated or magnesium-leached, both in nature and in building materials. In some varieties of chrysotile, minor substitution of silicon by Al^3+ may occur. Chrysotile is the most prevalent type of asbestos.

3.2.12 *cleavage*—the breaking of a mineral along one of its crystallographic directions.

3.2.13 *cleavage fragment*—a fragment of a crystal that is bounded in whole or in part by cleavage faces. Some cleavage fragments would be included in the fiber definition used in this method.

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

3.2.15 *d-value or interplanar spacing*—the perpendicular distance between identical adjacent and parallel planes of atoms in a crystal.

3.2.16 *decision value, n*—the structure count that must be exceeded to claim that a measurement represents a population of airborne structures that is different than the background population, which is established by analyzing blanks (see 3.2.9 and Practice D6620).

3.2.17 *electron diffraction*—techniques in electron microscopy, including selected area electron diffraction (SAED) and microdiffraction, by which the crystal structure of a specimen is examined.

3.2.18 *electron scattering power*—the extent to which a substance scatters electrons from their original courses.

3.2.19 *energy dispersive X-ray analysis*—measurement of the energies and intensities of X-rays by use of a solid state detector and multichannel analyzer system.

3.2.20 *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.

3.2.21 *field blank*—a filter cassette that has been taken to the sampling site, opened, and then closed. Such a filter is used to determine the background structure count for the measurement.

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

3.2.23 *fiber*—an elongated particle that has parallel or stepped sides. For the purposes of this test method, a fiber is defined as having an aspect ratio equal to or greater than 5:1 and a minimum length of 0.5 μm.

3.2.24 *fiber bundle*—a structure composed of parallel, smaller-diameter fibers attached along its length. A fiber bundle may exhibit diverging fibers at one or both ends.

[5] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[6] The non-asbestiform variations of the minerals indicated in 5.2.6 have different Chemical Abstracts Service (CAS) numbers.

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

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

3.2.27 *limit of detection*—the mean count for a population of structures that has been determined, based on a measurement or average of measurements, to be different than the background population of structures (see 3.2.16 and Practice D6620). The limit of detection may be restated in units of structures/L by multiplying the mean count by analytical sensitivity (see 3.2.4).

3.2.28 *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 nonfibrous particles.

3.2.29 *miller index*—a set of three integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes.

3.2.30 *PCM equivalent fiber*—a particle of aspect ratio that is greater than or equal to 3:1, is longer than 5 µm, and that has a diameter between 0.2 and 3.0 µm

3.2.31 *PCM equivalent structure*—a fibrous structure of aspect ratio that is greater than or equal to 3:1, is longer than 5 µm, and has a diameter between 0.2 and 3.0 µm.

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

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

3.2.34 *residual structure*—matrix or cluster material containing asbestos fibers that remains after accounting for the prominent component fibers or bundles, or both.

3.2.35 *serpentine*—a group of common rock-forming minerals having the nominal formula: $Mg_3Si_2O_5(OH)_4$.

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

3.2.37 *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.

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

3.2.39 *zone-axis*—the crystallographic direction parallel to the intersection edges of the crystal faces defining the crystal zone.

3.3 *Symbols:*

eV = electron volt
kV = kilovolt
L/min = liters per minute
µg = micrograms (10^{-6} g)
µm = micrometer (10^{-6} m)
nm = nanometer (10^{-9} m)
W = watt
Pa = Pascals

3.4 *Abbreviations:*

DMF = dimethyl formamide
ED = electron diffraction
EDXA = energy dispersive X-ray analysis
FWHM = full width, half maximum
HEPA = high-efficiency particle absolute
MCE = mixed cellulose ester; also refers to pure cellulose nitrate filters
PC = polycarbonate
PCM = phase contrast optical microscopy
ED = selected area electron diffraction
SEM = scanning electron microscope
STEM = scanning transmission electron microscope
TEM = transmission electron microscope
UICC = Union Internationale Contre le Cancer

4. Summary of Test Method

4.1 A sample of airborne particulate matter is collected by drawing a measured volume of air through either a capillary-pore polycarbonate membrane filter of maximum pore size 0.4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0.45 µm by means of a battery-powered or mains-powered pump. TEM specimens are prepared from polycarbonate filters by applying a thin film of carbon to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film of carbon that bridges the openings in the TEM specimen grid and that supports each particle from the original filter in its original position. Cellulose ester filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma to try to expose particles embedded in the collapsed 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 by a solvent extraction procedure.

4.2 The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative structure count on randomly-selected grid openings. In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fiber, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify each fiber unequivocally and fibers are classified according to the techniques that have been used to identify them. For each fiber, a simple code is used to record the manner in which it was classified. The fiber classification procedure is based on successive inspection of the morphology, the ED pattern, and the qualitative and quantitative EDXA. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone axis ED.

4.3 In addition to isolated fibers, ambient air samples often contain more complex aggregates of fibers, with or without other particles. Some particles are composites of asbestos

fibers with other materials. Individual fibers and these more complex structures are referred to as *asbestos structures*. A coding system is used to record the type of fibrous structure and to provide a description of each of these complex structures. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibers. The procedure permits a minimum required fiber identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fiber, and the degree of success is recorded for each fiber. The lengths and widths of all classified structures and fibers are recorded. The number of asbestos structures found on a known area of the microscope sample, together with the equivalent volume of air filtered through this area, is used to calculate the airborne concentration in asbestos structures/L of air.

5. Significance and Use

5.1 This test method is applicable to the measurement of airborne asbestos in a wide range of ambient air situations and for detailed evaluation of any atmosphere for asbestos structures. Most fibers in ambient atmospheres are not asbestos, and therefore, there is a requirement for fibers to be identified. Most of the airborne asbestos fibers in ambient atmospheres have diameters below the resolution limit of the light microscope. This test method is based on transmission electron microscopy, which has adequate resolution to allow detection of small thin fibers and is currently the only technique capable of unequivocal identification of the majority of individual fibers of asbestos. Asbestos is often found, not as single fibers, but as very complex, aggregated structures, which may or may not also be aggregated with other particles. The fibers found suspended in an ambient atmosphere can often be identified unequivocally if sufficient measurement effort is expended. However, if each fiber were to be identified in this way, the analysis would become prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate matter, some fibers cannot be positively identified as asbestos even though the measurements all indicate that they could be asbestos. Therefore, subjective factors contribute to this measurement, and consequently, a very precise definition of the procedure for identification and enumeration of asbestos fibers is required. The method defined in this test method is designed to provide a description of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. The test method is necessarily complex because the structures observed are frequently very complex. The method of data recording specified in the test method is designed to allow reevaluation of the structure-counting data as new applications for measurements are developed. All of the feasible specimen preparation techniques result in some modification of the airborne particulate matter. Even the collection of particles from a three-dimensional airborne dispersion on to a two-dimensional filter surface can be considered a modification of the particulate matter, and some of the particles, in most samples, are modified by the specimen preparation procedures. However, the procedures specified in this test method are designed to minimize the disturbance of the collected particulate material.

5.2 This test method applies to analysis of a single filter and describes the precision attributable to measurements for a single filter (see 13.1). Multiple air samples are usually necessary to characterize airborne asbestos concentrations across time and space. The number of samples necessary for this purpose is proportional to the variation in measurement across samples, which may be greater than the variation in a measurement for a single sample.

6. Apparatus

6.1 *Air Sampling Equipment and Consumable Supplies:*

6.1.1 *Filter Cassette,* 25 to 50-mm-diameter, commercially-manufactured, nonreusable, three-piece cassettes, with cowls in front of the filter surface, used for sample collection. Load the cassette with either a capillary pore polycarbonate filter of maximum pore size 0.4 µm or an MCE of maximum pore size 0.45 µm. Back either type of filter with a 5 µm pore size MCE, and support it by a cellulose back-up pad. Apply a shrink cellulose band or adhesive tape when the filters are in position to prevent air leakage. Ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

6.1.1.1 It is recommended that representative filters from the filter lot be analyzed as described in 10.7 for the presence of asbestos structures before any are used for air sample collection.

6.1.2 *Sampling Pump,* capable of a flow-rate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4.0 and 45.0 cm/s. The sampling pump used shall provide a stable air-flow through the filter. A constant flow or critical orifice-controlled pump meets these requirements. Use flexible tubing to connect the filter cassette (see 6.1.1) to the sampling pump.

6.1.3 *Stand,* used to hold the filter cassette at the desired height for sampling, and to isolate it from the pump vibrations.

6.1.4 *Flow Meter,* a calibrated flow meter with an appropriate range for the sampling flow rate used. The flow meter should be calibrated to a primary standard.

6.2 *Equipment for Analysis:*

6.2.1 *Transmission Electron Microscope*—A TEM operating at an accelerating potential of 80–120 kV, with a resolution better than 1.0 nm, and a magnification range of approximately 300 to 100 000 with the ability to obtain a direct screen magnification of about 100 000, shall be used for inspection of fiber morphology. This magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular. It is also required that the viewing screen of the microscope be calibrated such that the lengths and widths of fiber images down to 1 mm width can be estimated in increments of 1 mm regardless of fiber orientation. This requirement is often fulfilled through use of a fluorescent screen with calibrated gradations in the form of circles, such as the one shown in Fig. 1.

6.2.1.1 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. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If ED is used, the

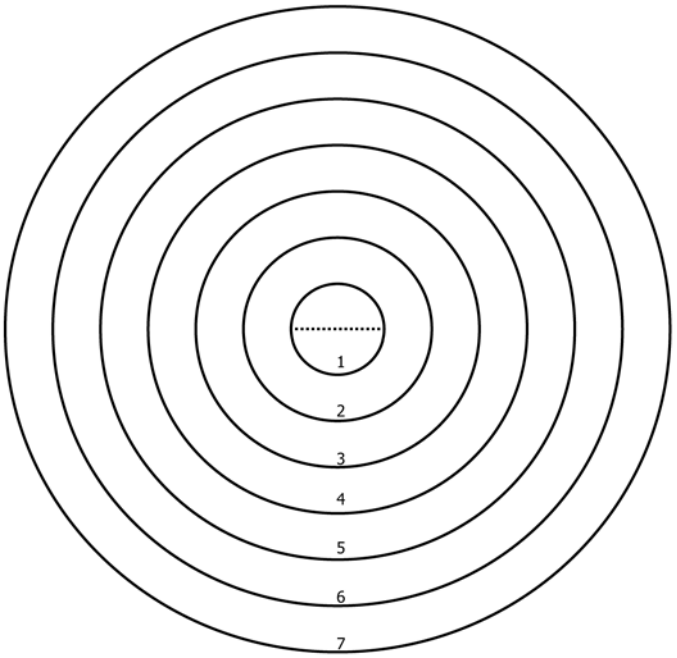


FIG. 1 Example of Calibration Markings on TEM Viewing Screen

performance of a particular instrument normally may be calculated using the following relationship:

$$A = 0.7854 \times (D/M + 2\,000 \times C_s\ \theta^3)^2 \quad (2)$$

where:

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

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

6.2.1.3 If zone-axis ED analyses of amphiboles are to be performed, the TEM shall incorporate a goniometer stage that permits the TEM specimen to be either:

(a) rotated through 360°, combined with tilting through at least +30 to –30° about an axis in the plane of the specimen; or

(b) tilted through at least +30 to –30° about two perpendicular axes in the plane of the specimen.

6.2.1.4 The analysis is greatly facilitated if the goniometer permits eucentric tilting, although tilting 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. If the goniometer does not permit eucentric tilting, a gold or other metal film must be evaporated on the sample so that ED patterns may be accurately calibrated.

6.2.1.5 The TEM shall have an illumination and condenser lens system capable of forming an electron probe smaller than 250 nm in diameter. It is recommended that an anticontamination trap be used around the specimen.

6.2.2 *Energy Dispersive X-ray Analyzer*—The TEM shall be equipped with an energy dispersive X-ray analyzer capable of achieving a resolution better than 180 eV (FWHM) on the MnKα 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 diameter. Solid state X-ray detectors are least sensitive in the low energy region, so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyzer shall yield, under routine analytical conditions, a background-subtracted NaKα 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. The peak/background ratio for this performance test shall exceed 1.0.

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

6.2.3 *Carbon Rod Sharpener,* to neck the carbon rods that allow the carbon to be evaporated on to the filters with a minimum of heating.

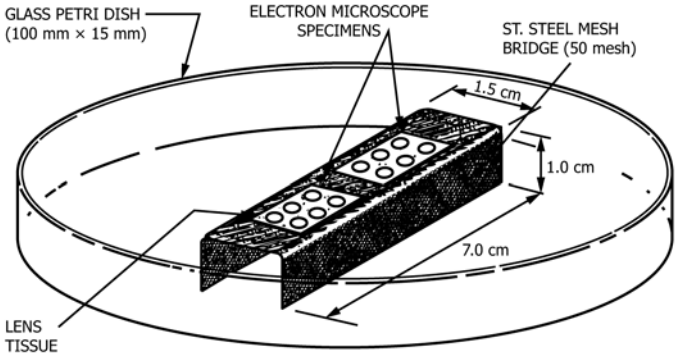
6.2.4 *Plasma Asher,* for preparation of TEM specimens from MCE filters. The plasma asher shall have a radio frequency power rating of 50 W or higher and be provided with a controlled, filtered oxygen flow. Admission of filtered air shall be through a valve to control the speed of air admission so that rapid air admission does not disturb particulate matter from the surface of the filter after the etching step.

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

6.2.5.1 Equip the vacuum coating unit with a mechanism that allows the rotating slide to be tilted also through an angle of approximately 45° during the coating procedure. A liquid nitrogen trap 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.

6.2.6 *Sputter Coater,* with a gold target used for deposition of gold onto 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.

6.2.7 *Solvent Washer* (Jaffe washer (**2**)), allows for 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. 2. Use either chloroform or 1-methyl-2-pyrrolidinone for dissolving polycarbonate filters, and use dimethyl formamide or acetone for dissolving MCE or cellulose nitrate filters. A



NOTE 1—Solvent is added until the meniscus contacts the underside of the stainless steel mesh.

FIG. 2 Example of Design of Solvent Washer (Jaffe Washer)

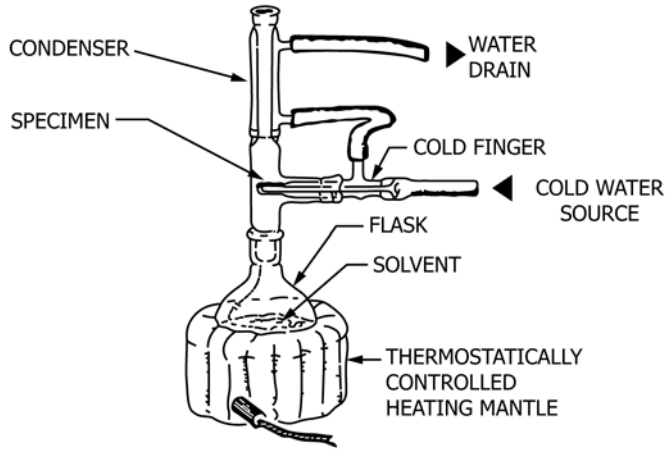


FIG. 3 Design of Condensation Washer

mixture of 20 % 1-2-diaminoethane and 80 % 1-methyl-2-pyrrolidinone may also be used to dissolve polycarbonate filters (**3**). The higher evaporation rates of chloroform and acetone require that a reservoir of 10 to 50 mL of solvent be used, which may need replenishment during the procedure. Because dimethyl formamide and 1-methyl-2-pyrrolidinone have lower vapor pressures, much smaller volumes of solvent may be used. Use the washer in a fume hood, and keep the petri dishes covered with their lids when specimens are not being inserted or removed during the solvent dissolution. Clean the washer before it is used for each batch of specimens.

6.2.8 *Condensation Washer,* used for more rapid dissolution of the filter polymer or for dissolving the filter polymer if difficulties are experienced. The washer consists of a flask, condenser, and cold finger assembly with a heating mantle and means for controlling the temperature. A suitable assembly is shown in Fig. 3. Use either acetone or chloroform as the solvent, depending on the type of filter.

6.2.9 *Slide Warmer or Oven,* for heating slides during the preparation of TEM specimens from MCE or cellulose nitrate filters, capable of maintaining a temperature of 65 to 70°C.

6.2.10 *Ultrasonic Bath,* for cleaning of apparatus used for TEM specimen preparation.

6.2.11 *Carbon Grating,* with approximately 2000 parallel lines per mm, used to calibrate the magnification of the TEM (see 6.2.1).

6.2.12 *Calibration Specimen Grids for EDXA,* TEM specimen grids prepared from dispersions of calibration minerals required for calibration of the EDXA system: crocidolite asbestos (NIST SRM 1866) and chrysotile asbestos.

6.3 *Reference Asbestos Samples,* for preparation of reference TEM specimens of the primary asbestos minerals. The UICC or NIST set of minerals are suitable for this purpose.

7. Reagents and Materials

7.1 *Reagents:* **Warning—**Use the reagents in accordance with the appropriate health and safety regulations. Review their Material Safety Data Sheets before use.

7.1.1 *Purity of Water*—Water shall be reagent water as defined by Type II of Specification D1193.

7.1.2 *Chloroform,* analytical grade, distilled in glass (preserved with 1 % (v/v) ethanol).

7.1.3 *1-Methyl-2-Pyrrolidinone,* analytical grade.

7.1.4 *Dimethyl Formamide,* analytical grade.

7.1.5 *Glacial Acetic Acid,* analytical grade.

7.1.6 *Acetone,* analytical grade.

7.1.7 *1-2-Diaminoethane,* analytical grade.

7.2 *Materials:*

7.2.1 *Copper Electron Microscope Grids, 200-mesh* TEM grids with grid openings of uniform size such that they meet the requirement of 10.6.3. Use grids with numerical or alphabetical indexing of individual grid openings to facilitate the relocation of individual grid openings for quality assurance purposes.

7.2.2 *Gold Electron Microscope Grids,* 200 mesh gold 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 they meet the requirement of 10.6.3. Use grids with numerical or alphabetical indexing of individual grid openings to facilitate the relocation of individual grid openings for quality assurance purposes.

7.2.3 *Carbon Rod Electrodes,* spectrochemically pure for use in the vacuum evaporator during carbon coating of filters.

7.2.4 *Disposable Tip Micropipettes,* 30 µL.

7.2.5 *Core Borer,* 7 mm.

7.2.6 *Routine Electron Microscopy Tools and Supplies,* such as fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, gummed paper reinforcement rings, lens tissue, gold wire, tungsten filaments, and other routine supplies.

8. Specimen Preparation Laboratory

8.1 Asbestos, particularly chrysotile, may be present in varying quantities in laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibers that 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. Perform all specimen preparation steps in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination yields results that will meet the requirements specified in 10.7. 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 in specimen preparation appear to be more important than the type of clean handling facilities in use. Carry out preparation of samples only after acceptable blank values have been demonstrated.

8.2 Do not perform activities involving manipulation of bulk asbestos samples in the same area as TEM specimen preparation because of the possibilities of contaminating the TEM specimens.

9. Sampling

9.1 See Terminology D1356 and Practice D1357 for general information on sampling and EPA Documents on AHERA **(4)** and Superfund **(5)** for information about sampling for asbestos.

9.2 Establish the desired analytical sensitivity for the analysis prior to sample collection. It is defined as that structure concentration corresponding to the detection of one structure in the analysis. For direct transfer methods of TEM specimen preparation the analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, and the area of the TEM specimen over which structures are counted. Select the sampling rate and the period of sampling to yield the required analytical sensitivity, as detailed in Table 1.

9.2.1 Collect air samples using cassettes as qualified in 10.7. Support the filter cassette on a stand (see 6.1.3) that is isolated from the vibrations of the pump (see 6.1.2) during sampling. Hold the cassette facing downwards vertically at a height of approximately 1.5 to 2.0 m above ground floor level, and connect it to the pump with a flexible tube.

9.2.2 Measure the sampling flow-rate at the front end of the cassette, both at the beginning and end of the sampling period, using a calibrated flow meter (6.1.4) temporarily attached to the inlet of the cassette (see 6.1.1). Use the mean value of these two measurements to calculate the total air volume sampled. If the difference in flow rate at the beginning and end of the sampling period is greater than 20 %, the result should be labeled as suspect or void due to sampling errors.

TABLE 1 Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity for a Collection Filter Area of 385 mm^2 and TEM Grid Openings of 85 µms (0.0072 mm^2)

Analytical Sensitivity	Volume of Air Sampled, L						
Structures/L	500	1000	1200	2000	3000	4000	5000
0.1	1066	533	444	267	178	134	107
0.2	533	267	223	134	89	67	54
0.3	356	178	148	89	60	45	36
0.4	267	134	112	67	45	34	27
0.5	214	107	89	54	36	27	22
0.7	153	77	64	39	26	20	16
1.0	107	54	45	27	18	14	11
2.0	54	27	23	14	9	7	6
3.0	36	18	15	9	6	5	4
4.0	27	14	14	7	5	4	4
5.0	22	11	13	6	4	4	4
7.0	16	8	7	4	4	4	4
10.0	11	6	5	4	4	4	4

9.2.2.1 If flow-meter contamination is suspected, clean and recalibrate the flow meter before use to avoid transfer of asbestos contamination from the flow meter to the sample being collected.

9.2.3 Monitor sampling pumps on a periodic basis during the entire sampling time. Place a cap over the open end of the cassette (6.1.1) after sampling, and store the cassette with the filter face-upwards for return to the laboratory. Include blank field filters, as described in 10.7, and process them through the remaining analytical procedures along with the samples.

9.2.4 Determine the analytical sensitivity S in structures/L as follows:

$$S = A_f / (A_g \times V \times K) \quad (3)$$

where:

A_f = area of sample filter exposed to the passage of air, mm^2,
A_g = mean area of TEM specimen grid openings, mm^2,
V = volume of air sampled, L, and
K = number of grid openings to be examined.

9.2.5 To achieve a particular analytical sensitivity when the total airborne dust levels are high, it may be necessary to collect low volumes of air and examine many grid openings.

10. Analysis

10.1 *Introduction:*

10.1.1 The techniques used to prepare TEM specimens are different for polycarbonate and cellulose ester filters. The preparation method to be used shall be either as described in 10.3 or 10.4, depending on the type of membrane filter used for air sampling. Cleaning of the sample cassettes before they are opened, preparation of the carbon evaporator, criteria for acceptable specimen grids, and the requirement for blank determinations are identical for the two preparation techniques. TEM examination, structure counting, fiber identification, and reporting of results are independent of the type of filter or preparation technique used.

10.1.2 The ability to meet the blank sample criteria is dependent on the cleanliness of equipment and supplies. Consider all supplies, such as microscope slides and glassware, as potential sources of asbestos contamination. Wash all glassware before it is used. Wash any tools or glassware that come into contact with the air sampling filters or TEM specimen preparations, both before use and between handling of individual samples. Use disposable supplies whenever possible.

10.2 *Cleaning of Sample Cassette*—Asbestos fibers can adhere to the exterior surfaces of air sampling cassettes (see 6.1.1), and these fibers can inadvertently be transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette before the cassette is taken into the clean facility or laminar flow hood.

10.3 *Direct Preparation of TEM Specimens from Polycarbonate Filters:*

10.3.1 *Selection of Filter Area for Carbon Coating*—Use a cleaned microscope slide to support representative portions of

polycarbonate filter during the carbon evaporation. Use double-coated adhesive tape to hold the filter portions to the glass slide. Take care not to stretch the polycarbonate filters during handling. Remove the polycarbonate filter from the sampling cassette (see 6.1.1), using freshly-cleaned tweezers, and place it on to a second cleaned glass microscope slide that is used as a cutting surface. Cut the filter by rocking the blade from the point, using a freshly-cleaned curved scalpel blade, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. Wash and dry the scalpel blade and tweezers between the handling of each filter. Identify the filter portions by writing on the glass slide.

10.3.2 *Carbon Coating of Filter Portions*—Place the slide holding the filter portions on the rotation-tilting device, approximately 100 to 120 mm from the evaporation source, and evacuate the evaporator chamber to a vacuum better than 0.013 Pa. Perform the evaporation of carbon in very short bursts, separated by a few seconds to allow the electrodes to cool.

10.3.2.1 If evaporation of carbon is too rapid, the strips of polycarbonate filter will begin to curl, and cross-linking of the surface will occur. This cross-linking produces a layer of polymer that is relatively insoluble in organic solvents, and it will not be possible to prepare satisfactory TEM specimens. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30 to 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. Ensure that the carbon film thickness is the minimum possible while retaining most of the grid openings of the TEM specimen intact.

10.3.3 *Preparation of the Jaffe Washer*—Place several pieces of lens tissue, as shown in Fig. 2, on the stainless steel bridge, and fill the washer (see 6.2.7) with chloroform (see 7.1.2) to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue. Alternatively, without using lens paper, fill the washer with 1-methyl-2-pyrrolidone (see 7.1.3) or a mixture of 20 % 1,2-diaminoethane (see 7.1.7) and 80 % 1-methyl-2-pyrrolidinone to a level where the meniscus contacts the underside of the mesh.

10.3.4 *Use of the Jaffe Washer with Chloroform*—Cut three 3-mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion, using a curved scalpel blade. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter onto the saturated lens tissue in the Jaffe washer. Place the three specimen grids from one sample on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for at least 8 h. It has been found that some lots of polycarbonate filters will not completely dissolve in the Jaffe washer, even after exposure to chloroform for as long as three days. This problem also occurs if the surface of the filter was overheated during the carbon evaporation.

10.3.4.1 *Condensation Washing*—Prepare TEM specimens by washing for approximately 1 h in a Jaffe washer (see 6.2.7), transfer the piece of lens tissue supporting the specimen grids to the cold finger of the condensation washer (see 6.2.8), which has achieved stable operating conditions using chloroform (see 7.1.2) as the solvent. Operate the washer for approximately 30 min after inserting the grids.

10.3.5 *Use of the Jaffe Washer with 1-Methyl-2-Pyrrolidinone*—Cut three 3-mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion, using a curved scalpel blade. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the stainless steel mesh in the Jaffe washer. Any number of separate grids may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for 2 to 6 h. After dissolution is complete, remove the stainless steel mesh from the Jaffe washer and allow the grids to dry. 1-methyl-2-pyrrolidinone evaporates very slowly. If it is required to dry the grids more rapidly, transfer the stainless steel bridge into another petri dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desirable to retain the water-soluble particle species on the TEM grids, ethanol may be used instead of distilled water for the second wash.

10.3.6 *Use of the Jaffe Washer with a Mixture of 20 % 1,2-Diaminoethane and 80 % 1-Methyl-2-Pyrrolidinone*—Cut three 3-mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion, using a curved scalpel blade. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the stainless steel mesh in the Jaffe washer. Any number of separate grids may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for 15 min. After dissolution is complete (15 min), remove the stainless steel mesh from the Jaffe washer and transfer the stainless steel bridge into another petri dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desirable to retain the water-soluble particle species on the TEM grids, ethanol may be used instead of distilled water for the second wash.

10.4 *Direct Preparation of TEM Specimens from Cellulose Ester Filters:*

10.4.1 *Selection of Area of Filter for Preparation*—Remove the filter from the filter cassette (see 6.1.1), using clean tweezers, and place it on a cleaned microscope slide. Cut out a portion of the filter with a clean, curved scalpel blade or remove a section (a plug) from any quadrant of the filter using a 7-mm cork borer. The cork borer must be wet-wiped each time a section is removed.

10.4.2 *Preparation of a Dimethyl Formamide/Glacial Acetic Acid Solution or Acetone Washer for Collapsing Cellulose Ester Filters*—Mix 35 mL of dimethyl formamide (see 7.1.4), and 15 mL of glacial acetic acid (see 7.1.5) with 50 mL of freshly-distilled water. Store this mixture in a clean bottle. The mixture is stable and suitable for use for up to three months after preparation. Alternatively, prepare a fusing dish from a glass petri dish and a metal screen bridge with a pad of five to six ashless paper filters and place in the bottom of the petri dish. Place the screen bridge on top of the pad and saturate the filter pads with acetone.

10.4.3 *Filter Collapsing Procedures:*

10.4.3.1 For the dimethyl formamide/glacial acetic acid solution procedure, place 15 to 25 µL of the collapsing solution per cm^2 of filter on a cleaned microscope slide, using a micropipette (see 7.2.4) with a disposable tip, and using the end of the pipette tip, spread the liquid over the area to be occupied by the filter portion. Place the filter portion, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20° 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 (see 6.2.9) at a temperature of 65 to 70°C, or in an oven at this temperature, for 10 min. 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.

10.4.3.2 For the acetone washer procedure, place the filter plug (particle side up) on a clean microscope slide. Affix the filter section to the slide with a gummed page reinforcement, or other suitable means. Label the slide with a glass scribing tool or permanent marker. Place the slide on top of the bridge in the petri dish and cover the dish. Wait approximately 5 min for the sample filter to fuse and clear.

10.4.4 *Plasma Etching of the Filter Surface*—Determine experimentally the optimum conditions and time for plasma etching for the recovery of fine chrysotile fibrils on 0.45-µm pore size MCE filters. Establish the conditions required in a particular plasma asher (see 6.2.4) using the procedure defined in Annex A1. Place the microscope slide holding the collapsed filter portions in the plasma asher, and etch for the time and under the conditions determined. Ensure that the correct conditions are used. Admit air slowly to the chamber after etching, and remove the microscope slide.

10.4.4.1 Adjust the air admission valve of the plasma asher so that the time taken for the chamber to reach atmospheric pressure exceeds 2 min. Rapid air admission may disturb particulate matter on the surface of the etched filter.

10.4.5 *Carbon Coating*—Carbon coat the microscope slide holding the collapsed filter portions as described in 10.3.2.

10.4.6 *Preparation of the Jaffe Washer*—Place several pieces of lens tissue on the stainless steel bridge, and fill the washer (see 6.2.7) with dimethyl formamide (7.1.4) or acetone to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

10.4.7 *Placing of Specimens into the Jaffe Washer*—Place the specimens in the Jaffe washer as described in 10.3.4. Specimens are normally cleared after approximately 1 h.

10.4.8 *Rapid Preparation of TEM Specimens from Cellulose Ester Filters*—An alternative washing procedure may be used to prepare TEM specimens from cellulose ester filters more rapidly than can be achieved by the Jaffe washing procedure. After the specimens have been washed in a Jaffe washer (see 6.2.7) for approximately 1 h, transfer the piece of lens tissue supporting the specimens to the cold finger of a condensation washer (see 6.2.8) operating with acetone as the solvent. Operate the condensation washer for approximately 30 min. This treatment removes all remaining filter polymer.

10.4.8.1 Do not use dimethyl formamide (see 7.1.4) in the condensation washer.

10.5 *Criteria for Acceptable TEM Specimen Grids:*

10.5.1 Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Examine the TEM specimen grid in the TEM at a magnification sufficiently low (300 to 1000) so that complete grid openings can be inspected. Reject the grid if:

10.5.1.1 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 filter;

10.5.1.2 The sample is overloaded with particulate matter. If the specimen grid exhibits more than approximately 10 % obscuration on the majority of the grid openings, designate the specimen as overloaded. This filter cannot be analyzed satisfactorily using the direct preparation methods because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA, and obscuration of fibers by other particulate matter may lead to underestimation of the asbestos structure count;

10.5.1.3 The particulate matter deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate matter deposits on the specimen are obviously not uniform from one grid opening to the next, designate the specimen as nonuniform. This condition is a function either of the air sampling conditions or of the fundamental nature of the airborne particulate matter. Satisfactory analysis of this filter may not be possible unless a large number of grid openings are examined;

10.5.1.4 The TEM grid is too heavily loaded with fibrous structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7000 structures/mm^2, or

10.5.1.5 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 structure count.

10.5.2 If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, apply an additional carbon coating to the carbon coated filter, and prepare new specimen grids. The larger particles can often be

supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analyzed using the direct preparation methods.

10.5.3 If one or more of the conditions described in 10.5.1.2 – 10.5.1.5 exists, it may not be possible to analyze the sample by this method.

10.6 *Procedure for Structure Counting by TEM:*

10.6.1 *Introduction*—The examination consists of a count of asbestos structures that are present on a specified number of grid openings. Fibers within structures are classified into groups on the basis of morphological observations, ED patterns, and EDXA spectra. The total number of structures to be counted depends on the statistical precision desired. The precision of the structure count depends not only on the total number of structures counted, but also on their uniformity from one grid opening to the next. Additional structure counting will be necessary if greater precision is required. In the absence of asbestos structures, the area of the TEM specimen grids to be examined depends on the analytical sensitivity required.

10.6.2 So that the estimate of the structure density on the sampling filter shall not be based on the small area represented by one specimen grid, examine the grid openings on at least two of the three specimen grids prepared. Then combine the results in the calculation of the structure density. Make structure counts at a magnification of approximately 20 000, and terminate it at the end of the examination of the grid opening on which the 100th asbestos structure is observed, except continue the count until a minimum of four grid openings have been examined. Otherwise, continue the structure count to that number of grid openings at which the specified analytical sensitivity has been achieved.

10.6.2.1 The normal range for the number of grid openings examined is from 4 to 20. If insufficient air has been sampled through the filter, Eq 4 may indicate that an impractically large number of grid openings should be examined. When this situation occurs, a larger value of analytical sensitivity may have to be accepted.

10.6.3 *Measurement of Mean Grid Opening Area*—Measure the mean grid opening area for the type of TEM specimen grids in use. Ensure that the relative standard deviation of the mean of ten openings selected from ten grids is less than 5 %. As an optional procedure, or if the 5 % relative standard deviation criterion cannot be demonstrated, measure the dimensions of each grid opening examined in the TEM at a calibrated magnification.

10.6.4 *TEM Alignment and Calibration Procedures*—Align the TEM (see 6.2.1) according to instrumental specifications before structure counting is performed. Calibrate the TEM and EDXA system according to the procedures in Annex A2.

10.6.5 *Determination of Stopping Point*—Before structure counting is begun, calculate the area of specimen to be examined in order to achieve the selected analytical sensitivity. Determine the maximum number of grid openings to be examined from the formula:

$$k = A_f/(A_g \times V \times S) \quad (4)$$

where:

k = number of grid openings to be examined. Round k up to the next highest integer,
A_f = area of sample filter in mm^2,
A_g = area of TEM specimen grid opening in mm^2,
V = volume of air sampled in L, and
S = required analytical sensitivity in structures/L.

10.6.6 *General Procedure for Structure Counting and Size Analysis*—Two specimen grids prepared from the filter will be used in the structure count. Several grid openings from each grid will be selected at random, and the data combined in the calculation of the results. Use a form containing, at a minimum, those elements shown in Fig. 4 to record the data. Insert the first specimen grid into the TEM (see 6.2.1).

10.6.6.1 Insert the grid into the specimen holder in a standard and relocatable orientation with the grid bars parallel and perpendicular to the axis of the specimen holder to facilitate quality assurance measurements that require reexamination of the same grid opening by different microscopists. This will provide scan directions parallel to the edges of the grid opening. Ensure that all microscopists begin scanning at the same starting point on the grid opening and that they use similar scan patterns. This procedure permits rapid relocation of fibrous structures for further examination, if necessary.

10.6.6.2 Select a typical grid opening and set the screen magnification to the calibrated value (approximately 20 000). Adjust the sample height until the features in the center of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero. Record the number or letter used to identify the grid in Column 1 of the data recording form. Record the identification of the particular grid opening in Column 2. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for fibers, until the opposite side of the grid opening is encountered. Move the image by a predetermined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in Fig. 5.

10.6.6.3 When a fibrous structure is detected, assign a sequential number to the primary structure in Column 3, perform the identification procedures required as detailed in Annex A5, and enter the appropriate compositional classification on the structure counting form in Column 5. Assign a morphological classification to the structure according to the procedures in Annex A4, and record this classification in Column 6. Measure on the TEM viewing screen the length and width of the image of the primary structure in mm, and record these measurements in Columns 7 and 8. For a disperse cluster or matrix, assign a compositional classification and a morphological classification to each structure component, measure the length and width, and enter the data in Columns 4 through 8. Use Column 4 of the data recording form to tabulate the sequential number of total structures, taking into account structure components. If nonasbestos fibers are observed, note their presence and type, if known.

AIRBORNE ASBESTOS BY DIRECT TRANSFER TEM

TEM ASBESTOS STRUCTURE COUNT

Report Number: _____ **Air Volume:** _____ **L**

Sample Number: _____ **Sample filter area:** _____ **mm^2**

File Name: _____

Sample Description: _____

_____

_____

Preparation data: _____ **By:** _____ **Magnification:** _____

Analysis date: _____ **By:** _____ **Grid opening dimension:** _____ **μm**

Computer entry date: _____ **By:** _____ **Level of analysis (C):** _____

(A): _____

Grid	Grid Opening	Structures Primary	Structures Total	Class	Structure	Length (mm)	Width (mm)	Comments

FIG. 4 Example of Structure Counting Form

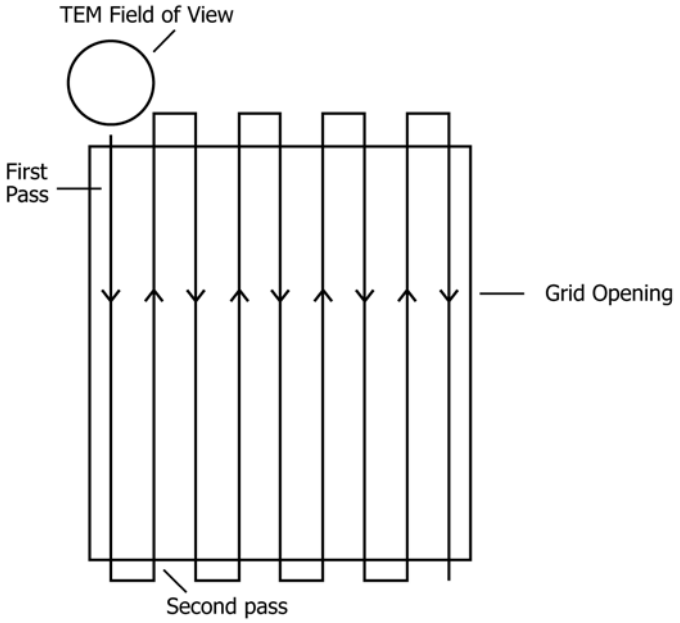


FIG. 5 Example of Scanning Procedure for TEM Specimen Examination

10.6.6.4 After a fibrous structure has been examined and measured, relocate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause structures to be overlooked or counted twice. Continue the examination until the completion of the grid opening on which the 100th asbestos structure has been recorded, or until the number of grid openings required to achieve the specified analytical sensitivity, calculated according to 10.6.5, have been examined, whichever occurs first. Draw the data approximately equally from a minimum of two grids. Count fibrous structures on a minimum of four grid openings regardless of the value calculated according to 10.6.5.

10.6.7 *Measurement of Concentration for Asbestos Fibers and Bundles Longer Than 5 µm*—Give consideration to improving the measurement accuracy of asbestos fibers and bundles longer than 5 µm by additional examination at a lower magnification, taking account of only the longer fibers and bundles. Perform this extended examination for fibers and bundles longer than 5 µm in accordance with the procedures described in Annex A5. Use a magnification of approximately 10 000 for counting of all asbestos fibers and bundles longer than 5 µm, or approximately 5000 if only fibers and bundles within the diameter range 0.2 to 3.0 µm are to be counted. Continue the count until completion of the grid opening on which 100 fibers and bundles have been recorded, or until sufficient area of the specimen has been examined to achieve the desired analytical sensitivity.

10.6.7.1 Report only those structures that are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals in either the original or the extended TEM examination. Do not include other materials, for example, gypsum (see Appendix X2), cellulose fibers, and filter artifacts, such as undissolved filter strands in the fiber count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

10.7 *Blank and Quality Control Determinations*—Analyze a minimum of two unused filters from each filter lot of 100 filters before air samples are analyzed to determine the mean asbestos structure count. Reject the filter lot if the mean count for all types of asbestos structures is found to be more than ten structures/mm^2, or if the mean fiber count for asbestos fibers and bundles longer than 5 µm is more than 0.1 fiber/mm^2.

10.7.1 Establish a continuous program of blank measurements to ensure that contamination by extraneous asbestos fibers during specimen preparation is insignificant compared with the results reported on samples. Process at least one field blank along with each batch of samples. In addition, include a portion of an unused filter with every group of samples prepared on one microscope slide. Should the analysis of any field blank (or the filter section blank processed with each microscope slide) result in a count exceeding a structure density of ten total structures/mm^2 or a count of asbestos fibers and bundles longer than 5 µm greater than 0.1 fiber/mm^2, corrective action will be required. Concentrations derived for samples in batches associated with nonconforming field blanks (or for sample filter sections on microscope slides associated with nonconforming blanks) should be labeled with a qualifier indicating that these values are associated with elevated background counts observed in blank measurements. Simultaneously, a review of field and laboratory procedures should be conducted to evaluate the need for adjustments and corrections that may be required to prevent extraneous sources of contamination from affecting future sampling and analysis results.

10.7.2 Initially, and also at intervals afterwards, ensure that samples of known asbestos concentrations can be analyzed satisfactorily. Since there is a subjective component in the structure-counting procedure, make recounts of some specimens by different microscopists to minimize the subjective effects. Such recounts provide a means of maintaining comparability between counts made by different microscopists. Characterize variability between and within microscopists and between laboratories. Constitute approximately 10 % of the analyses in the quality assurance measurements. Ensure that repeat results do not differ at the 5 % significance level. The manner for establishing the 5 % level of significance for within and between microscopist recounts and between laboratory recounts should be documented as part of each laboratory's written quality assurance program plan and should be maintained in files along with the record for each such recount. Based on the recounts in which each microscopist participates, control charts should also be established and maintained to track the performance of each individual microscopist in the laboratory. Based on such control charts, microscopists who exhibit persistent bias relative to other microscopists within the laboratory or relative counts from other laboratories should be required to undergo retraining as part of a regular program that is also to be documented and incorporated as part of the laboratory's written quality assurance program plan.

10.8 *Calculation of Results*—Calculate the results using the procedures detailed in Annex A6 according to the level of analysis specified. Specify the level of analysis prior to the TEM examination of the specimens. Before the results are

calculated, include the compositional and morphological classifications in the result. Conduct the chi-squared uniformity test using the number of primary asbestos structures found on each grid opening, prior to the application of the cluster and matrix counting criteria. Determine the concentration result using the numbers of primary asbestos structures. Additional reporting information is illustrated in Fig. 6.

11. Quality Assurance

11.1 *Introduction:*

11.1.1 Use a continuous quality control program in conjunction with this analytical method. Include in the quality control program the use of standard samples, blank samples, and both inter- and intra-laboratory analyses.

AIRBORNE ASBESTOS BY DIRECT TRANSFER TEM

SAMPLE ANALYSIS INFORMATION

Laboratory name **Report Number** **Date**

Method: ASTM _____ Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by TEM Direct Transfer

Sample: 456 Beard Street
Springfield, N. Carolina
Exterior sample 1991-09-09

Air volume:	2150.0 L
Area of collection filter:	385.0 mm^2
Level of analysis (chrysotile):	CD or CMQ
Level of analysis (amphibole):	ADQ
Magnification used for fiber counting:	20500
Aspect ratio for fiber definition:	5:1
Mean dimension of grid openings:	95.4 µm
Initials of Analyst:	JMW
Number of grid openings examined:	10
Analytical Sensitivity:	1.968 structures/L
Number of primary asbestos structures:	13
Number of asbestos structures counted:	26
Number of asbestos structures >5 µm:	7
Number of asbestos fibers and bundles >5 µm:	10
Number of PCM equivalent asbestos structures:	3
Number of PCM equivalent asbestos fibers:	5
Concentration of Asbestos (Chrysotile)	43.3 structures/L
Concentration of Asbestos (Amphibole)	7.9 structures/L
Lower 95% Confidence Limit (Chrysotile)	27.1 structures/L
Upper 95% Confidence Limit (Chrysotile)	65.6 structures/L
Lower 95% Confidence Limit (Amphibole)	2.1 structures/L
Upper 95% Confidence Limit (Amphibole)	20.2 structures/L
Compositional Data for Amphibole	Na,Mg,Si,Fe

FIG. 6 Example of Format for Reporting Sample and Preparation Data

11.2 *Interferences and Limitations of Fiber Identification*—Unequivocal identification of every chrysotile fiber is not possible, due to both instrumental limitations and the nature of some of the fibers. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the fiber selected. However, there is a possibility of misidentification of fibers for which both the morphologies and the ED patterns are reported on the basis of visual inspection only. The only significant possibilities of misidentification occur with halloysite, vermiculite scrolls, or palygorskite, all of which can be discriminated from chrysotile by the use of EDXA and by observation of the 0.73-nm (002) reflection of chrysotile in the ED pattern.

11.3 As in the case of chrysotile fibers, complete identification of every amphibole fiber is not possible due to instrumental limitations and the nature of some of the fibers. Moreover, complete identification of every amphibole fiber is not practical due to the limitations of both time and cost. Particles of a number of other minerals having compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone-axis ED techniques. However, the requirement for quantitative EDXA measurements on all fibers as support for the random orientation ED technique makes misidentification very unlikely, particularly when other similar fibers in the same sample have been identified as amphibole by zone-axis methods. The possibility of misidentification is further reduced with increasing aspect ratio since it is rare for the minerals with which amphibole may be confused to display an asbestiform habit (**6**).

12. Report

12.1 Include in the test report the following information:

12.1.1 Reference to Test Method D6281.

12.1.2 Identification of the sample.

12.1.3 Air volume if provided (if not provided, this absence must be noted in the report).

12.1.4 The date of the analysis.

12.1.5 The identify of the analyst.

12.1.6 Any procedure used but not specified in this test method or regarded as an optional procedure.

12.1.7 A complete listing of the structure counting data (see Fig. 7). Include the following data: grid opening number, structure number, identification category, structure type, length and width of the structure in µm, and any comments concerning the structure.

12.1.8 A statement of the minimum acceptable identification category and the maximum identification category attempted (refer to Table A4.1 and Table A4.2).

12.1.9 A statement specifying which identification and structure categories have been used to calculate the concentration values.

12.1.10 Separate concentration values for chrysotile and amphibole structures, expressed in asbestos structures/L.

12.1.11 The 95 % confidence interval limits for the concentration values, expressed in asbestos structures/L.

12.1.12 The analytical sensitivity, expressed in asbestos structures/L.

12.1.13 Optional: the limit of detection, expressed in asbestos structures/L.

12.1.14 Compositional data for the principal varieties of amphibole, if present.

12.1.15 Optional items 12.1.7 – 12.1.13 for asbestos fibers and bundles longer than 5 µm.

12.1.16 Optional items 12.1.7 – 12.1.13 for PCM equivalent asbestos fibers and bundles.

12.1.17 An example of a suitable reporting format for the structure counting data is shown in Fig. 7.

13. Precision and Bias[7]

13.1 *Precision*—The precision of this test method is based on an interlaboratory study conducted in 2004 and 2005. Each of the ten laboratories tested two different samples for asbestos fiber count. Every "test result" represents an individual determination. Each laboratory analyzed and submitted two replicate test results (from one operator) for each material. Except for the use of only two materials, Practice E691 was followed for the design and analysis of the data.

13.1.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the *r* value for that material; *r* is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

13.1.1.1 Repeatability limits are listed in Table 2.

13.1.2 *Reproducibility (R)*—Two test results shall be judged not equivalent if they differ by more than the *R* value for that material; *R* is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

13.1.2.1 Reproducibility limits are listed in Table 2.

13.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

13.1.4 Any judgment in accordance with statements 13.1.1 and 13.1.2 would have an approximate 95 % probability of being correct.

13.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

13.3 The precision statement was determined through statistical examination of 34 results, from ten laboratories, on two materials. These samples were described as follows:

13.3.1 *Sample A*—A chrysotile asbestos fiber-containing air filter cassette. The filters were prepared by the New York State Health Department using a chrysotile asbestos fiber air dispersion. The chrysotile was deposited simultaneously on 109 filter cassettes in an aerosol-generation chamber that pulled air through each cassette at a flow rate of 1.0 litre per minute. This produced filters with loadings of approximately 300 structures per mm^2.

[7] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D22-1031. Contact ASTM Customer Service at service@astm.org.

AIRBORNE ASBESTOS BY DIRECT TRANSFER TEM

SAMPLE ANALYSIS INFORMATION (Page 2 and Higher)

Laboratory Name **Report Number** **Date**

Sample: 456 Beard Street
Springfield, N. Carolina
Exterior sample 1991-09-09

TEM ASBESTOS STRUCTURE COUNT - RAW DATA

Grid	Grid Opening	Structures Primary	Structures Total	Identification*	Structure Type	Length µm	Width µm	Comments
A	F4-4	1	1	CD	F	1.7	0.045	
		2	2	CMQ	B	3.6	0.09	
		3	3	ADQ	F	4.0	0.15	Crocidolite
	E3-6	4	4	CD	MC+0	3.5	1.3	
	E5-1	5		CD	MD43	7.5	5.0	
			5	CD	MB	7.7	0.30	
			6	CMQ	MF	5.6	0.045	
			7	CD	MB	5.1	0.30	
			8	CD	MF	1.7	0.045	
B	F5-1	6		CD	CD+0	6.5	3.0	
			9	CD	CB	3.5	0.15	
			10	CD	CF	3.5	0.045	
			11	CMQ	CR+0	2.6	1.9	
	G5-1	7		CD	CD31	6.1	3.2	
			12	CD	CB	5.6	0.3	
			13	CMQ	CF	4.0	0.045	
			14	CMQ	CB	3.2	0.090	
	E4-4	8	15	CD	B	1.5	0.23	
		9	16	AD	F	8.7	0.15	
C	G4-4	10		CMQ	CD42	25.	5.6	
			17	CMQ	CB	15.	0.15	
			18	CMQ	CF	9.4	0.045	
			19	ADQ	CF	3.6	0.30	Tremolite
			20	CM	CF	4.2	0.045	
	E4-4			No Fibers				
	E5-6	11		ADQ	CD+3	9.4	2.5	
			21	ADQ	CF	7.1	0.30	Amosite
			22	ADQ	CF	6.2	0.10	Crocidolite
			23	CM	CB	5.1	0.2	
			24	CM	CR+0	3.3	1.8	
	F4-1	12	25	CMQ	MC10	3.7	2.1	
		13	26	CD	CC+0	7.4	0.5	

*Identification codes listed in Tables A1 and A2

FIG. 7 Example of Format for Reporting of Structure Counting Data

TABLE 2 Asbestos Fibers (*Number*)

Material	Average[A] $\bar{x}$	Standard Deviation $s_{\bar{x}}$	Repeatability Standard Deviation S_r	Reproducibility Standard Deviation S_R	Repeatability Limits *r*	Reproducibility Limits *R*
A	103	54	31	58	85	162
B	333	52	31	57	88	159

[A] The average of the laboratories' calculated averages.

13.3.2 An amosite asbestos fiber-containing air filter cassette. The filters were prepared by the New York State Department of Health using an amosite fiber air dispersion. The amosite was deposited simultaneously on 109 filter cassettes in an aerosol-generation chamber that pulled air through each cassette at a flow rate of 1.0 litre per minute.

14. Keywords

14.1 air; ambient air; amphibole; asbestos; chrysotile; electron microscopy; indoor atmospheres; microscopy

ANNEXES

(Mandatory Information)

A1. DETERMINATION OF OPERATING CONDITIONS FOR THE PLASMA ASHER

A1.1 Introduction

A1.1.1 During preparation of TEM specimens from an MCE or cellulose nitrate filter, the spongy structure of the filter is collapsed into a thinner film of polymer by action of a solvent. Some of the particles on the surface of the original filter become completely buried in the polymer, and the specimen preparation procedure incorporates a plasma etching step to oxidize the surface layer of the polymer. Particles buried by the filter collapsing step are then exposed so that they can become affixed subsequently in the evaporated carbon film without altering their position on the original filter. The amount of etching is critical, and individual ashers vary in performance. Therefore, calibrate the plasma asher for a known amount of etching of the surface of the collapsed filter. This is performed by adjusting the radio frequency power output and the oxygen flow rate, and by measuring the time taken to completely oxidize an uncollapsed 25-mm diameter cellulose ester filter of the same type and pore size as those used in the analysis.

A1.2 Procedure

A1.2.1 Place a microscope slide containing a collapsed, 25-mm diameter cellulose ester filter of the type being used into the center of the asher chamber. Close the chamber and evacuate to a pressure of 40 Pa, while admitting oxygen to the chamber at a rate of 8 to 20 mL/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Measure the time required for complete oxidation of the filter. Determine the operating parameters that result in complete oxidation of the filter in a period of 15 min. Use these operating parameters to etch a preweighed, collapsed filter for a period of 8 min. Weight the filter after etching. Adjust the parameters and retest until a 1 to 10 % weight loss is achieved. The final acceptance of the etched filter is dependent on its appearance in the TEM.

A1.2.1.1 Plasma oxidation at high radio-frequency powers will cause the filter to shrink and curl, followed by sudden violent ignition. At lower powers, the filter will remain in position and will become slowly thinner until it becomes nearly transparent. Adjust the radio-frequency power such that violent ignition does not occur. When multiple filters are etched, the rate of etching is reduced; calibrate the system accordingly.

A2. CALIBRATION PROCEDURES

A2.1 Calibration of the TEM

A2.1.1 *Calibration of TEM Screen Magnification*—Align the electron microscope according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica. Adjust the specimen height to the eucentric position before carrying out the calibration. Measure the distance on the fluorescent viewing screen occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Repeat the calibration after any instrumental maintenance or change of operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates or film. The ratio between these is a constant value for the particular model of TEM.

A2.1.2 *Calibration of ED Camera Constant*—Calibrate the camera constant of the TEM when used in ED mode. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film with the specimen adjusted to the eucentric position and select ED conditions. Adjust the objective lens current to optimize the pattern obtained, and measure, either on the fluorescent viewing screen or on a recorded image, the diameters of the innermost two rings. Calculate the radius-based camera constant, $\lambda \times L$, for both the fluorescent screen and the photographic plate or film, from the relationship:

$$\lambda \times L = (a \times D)/(2.0 \times ((h^2 + k^2 + l)^{2½})) \quad \text{(A2.1)}$$

where:

λ = the wavelength of the incident electrons in nm,
L = the camera length in mm,
a = the unit cell dimension of gold in nm (0.40786 nm), and
D = the diameter of the (hkl) diffraction ring in mm.

Using gold as the calibration material, the radius-based camera constant is given by:

$\lambda \times L = 0.11774 \times D$ mm.nm (smallest ring), and
$\lambda \times L = 0.10197 \times D$ mm.nm (second ring).

A2.2 Calibration of the EDXA System

A2.2.1 Perform an energy calibration of the EDXA system for a low-energy and high-energy peak regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10 % of the elemental concentration, to be obtained from EDXA spectra of reference silicate minerals involving the elements Na, Mg, Al, Si, K, Ca, Mn, and Fe, and applicable certified reference materials. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination that meets the instrumental specifications of 6.2.1 and 6.2.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection are that they be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine and that individual small fragments of the minerals are homogeneous in composition within a few percent.

A2.2.2 Compositions of reference materials can be determined by electron microprobe analysis or chemical methods. Crush fragments of the same selected mineral standards and prepare filters by dispersal of the crushed material in water and immediate filtration of the suspensions. Prepare TEM specimens from these filters by the procedures described in Section 10. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable compositional results can be obtained from different instruments.

A2.2.2.1 The microprobe analyses of the mineral standards are made by conventional techniques that can be found in the bibliography. First embed the mineral in a mount of polymethyl methacrylate or epoxy resin. Then grind and polish the mount to achieve a flat, polished surface of the mineral fragment. Analyze this surface using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take account of the water concentration in the minerals, which in the case of chrysotile amounts to 13 % by weight. This water content may vary due to losses in the vacuum system.

A2.2.3 Filter the aqueous suspensions of the mineral standards immediately after preparation since alkali and alkali earth metals may be partially leached from minerals containing these elements.

A2.2.4 Express the results of the electron microprobe analyses as atomic or weight percentage ratios relative to silicon. Use the X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, to calculate the relationship between peak area ratio and atomic or weight percentage ratio. The technique was described by Cliff and Lorimer (**7**).

A2.2.5 The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus mass absorption and fluorescence effects are negligible. In a silicate mineral specimen containing element i, use the following relationship to perform quantitative analyses in the TEM:

$$\frac{C_i}{C_{Si}} = k_i \; X \; \frac{A_i}{A_{Si}} \quad \text{(A2.2)}$$

where:

C_i = the concentration or atomic proportion of element i,
C_{Si} = the concentration or atomic proportion of silicon,
A_i = the elemental integrated peak area for element i,
A_{Si} = the elemental integrated peak area for silicon, and
k_i = k-ratio for element i relative to silicon.

A2.2.6 For a particular instrumental configuration and a particle size, the value of k_i is constant.

A2.2.7 To incorporate the correction for the particle size effect on peak area ratios as described by Small, et al. (**8**), extend the Cliff and Lorimer technique by obtaining separate

values of the constant k_i for different ranges of fiber diameter. Take 20 EDXA measurements for each range of fiber diameter. Suitable ranges of fiber diameter are <0.25 µm, 0.25 to 0.5 µm, and >1.0 µm.

A2.2.8 Insert the TEM grid into the TEM, obtain an image at the calibrated higher magnification of about 20 000, and adjust the specimen height to the eucentric point. If the X-ray detector is a side-entry variety, tilt the specimen towards the X-ray detector. Select an isolated fiber or particle less than 0.5 µm in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centered on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. Ensure that all background-subtracted peak areas used for calibration exceed 400 counts.

A2.2.9 Repeat this procedure for 20 particles of each mineral standard. Reject analyses of any obviously foreign particles. Calculate the arithmetic mean concentration to peak area ratio, k_i (k-ratio), for each specified element of each mineral standard, and for each of the fiber diameter ranges. Perform periodic routine checks to ensure that there has been no degradation of the detector performance. Use these k-ratios to calculate the elemental concentrations of the unknown fibers, using the Cliff and Lorimer relationship.

A3. STRUCTURE COUNTING CRITERIA

A3.1 Introduction

A3.1.1 In addition to isolated fibers, other assemblages of particles and fibers frequently occur in air samples. Groupings of asbestos fibers and particles, referred to as *asbestos structures*, are defined as fiber bundles, clusters, and matrices. The numerical result of a TEM examination depends strongly on whether the analyst assigns such an assemblage of fibers as a single entity or as the estimated number of individual fibers that form the assemblage. It is therefore important that a logical system of counting criteria be defined so that the interpretation of these complex structures is the same for all analysts and so that the numerical result is meaningful. Imposition of specific structure-counting criteria generally requires that some interpretation be made of each asbestos structure found. It is the intention of this test method that a clear separation shall be made between recording of structure counting data and later interpretation of those data. The system of coding specified in this test method permits a morphological description of the structures to be recorded in a manner suitable for later interpretation, if necessary, by a range of different criteria without the necessity for reexamination of the specimens. In particular, the coding system is designed to permit the recording of the dimensions of each complex fibrous structure and also whether these structures contain fibers longer than 5 µm. This approach permits later evaluations of the data to include considerations such as particle respirability and comparisons with historical indices of asbestos exposure. Examples of the various types of morphological structure, and the manner in which these are recorded are shown in Fig. A3.1.

A3.2 Structure Definitions and Treatment

A3.2.1 Designate each fibrous structure that is a separate entity as a primary structure, for example, as a fiber, bundle, cluster, or matrix.

A3.2.1.1 *Fiber*—Define any particle with parallel or stepped sides, with a minimum length of 0.5 µm, and with an aspect ratio of 5:1 or greater, as a fiber. For chrysotile asbestos, define the single fibril as a fiber. Assign a fiber with stepped sides a width equal to the average of the minimum and maximum widths. Use this average as the width in determination of the aspect ratio.

A3.2.1.2 *Bundle*—Define grouping composed of apparently attached parallel fibers as a bundle, with a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may be any value provided that it contains individual constituent fibers having aspect ratios equal to or greater than 5:1. Bundles may exhibit diverging fibers at one or both ends.

A3.2.1.3 *Cluster*—Define an aggregate of two or more randomly oriented fibers, with or without bundles, as a cluster. Clusters occur as two varieties:

(a) Compact Cluster (Type D), a disperse and open network in which one of the individual fibers or bundles cannot be unambiguously determined.

(b) Compact Cluster (Type C), a complex and tightly bound network, in which one or both ends of each individual fiber or bundle are obscured, such that the dimensions of individual fibers and bundles cannot be unambiguously determined.

(c) In practice, clusters can occur in which the characteristics of both types of cluster occur in the same structure. Where this occurs, assign the structure as a disperse cluster, and then follow a logical procedure by recording structure components in accordance with the counting criteria. The procedure for treatment of clusters is illustrated by examples in Fig. A3.2.

A3.2.1.4 *Matrix:*

(*a*) One or more fibers, or fiber bundles, may be attached to, or partially concealed by a single particle or group of overlapping nonfibrous particles. Define this structure as a matrix. The TEM image does not discriminate between particles that are attached to fibers and those that have by chance overlapped in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibers on the filter.

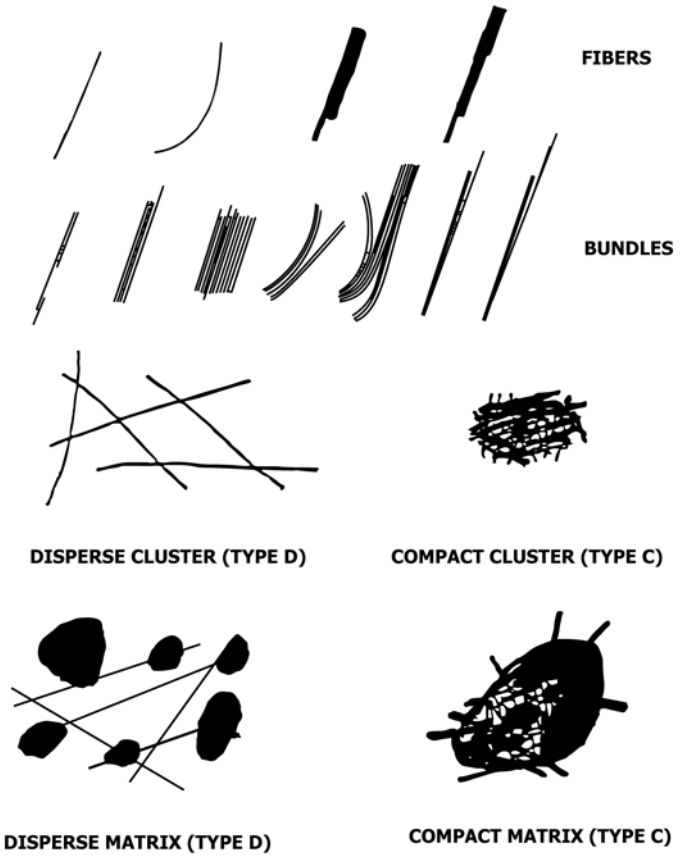


FIG. A3.1 Fundamental Morphological Structure Types

(*b*) Since a matrix structure may involve more than one fiber, define in detail how matrices are counted. Matrices exhibit different characteristics, and two types can be defined:

(*1*) *Disperse Matrix (Type D),* a structure consisting of a particle or linked group of particles, with overlapping or attached fibers or bundles in which at least one of the individual fibers or bundles can be separately identified and its dimensions measured;

(*2*) *Compact Matrix (Type C)* a structure consisting of a particle or linked group of particles, in which fibers or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibers and bundles cannot be unambiguously determined.

(*c*) In practice, matrices can occur in which the characteristics of both types of matrix occur in the same structure. Where this occurs, assign the structure as a disperse matrix, and then follow a logical procedure by recording structure components according to the counting criteria. Examples of the procedure that shall be followed are shown in Fig. A3.3.

A3.2.2 *Asbestos Structure Larger than 5 µm,* any fiber, bundle, cluster, or matrix for which the largest dimension exceeds 5 µm. Asbestos structures larger than 5 µm do not necessarily contain asbestos fibers or bundles longer than 5 µm.

A3.2.3 *Asbestos Fiber or Bundle Longer Than 5 µm,* an asbestos fiber of any width, or bundle of such fibers, that has a length exceeding 5 µm.

A3.2.4 *PCM Equivalent Structure,* any fiber, bundle, cluster, or matrix with an aspect ratio of 3:1 or greater, longer than 5 µm, and that has a diameter between 0.2 mm and 3.0 µm. PCM equivalent structures do not necessarily contain fibers or bundles longer than 5 µm, or PCM equivalent fibers.

A3.2.5 *PCM Equivalent Fiber,* any particle with parallel or stepped sides, with an aspect ratio of 3:1 or greater, longer than 5 µm, and with a diameter between 0.2 µm and 3.0 µm. For chrysotile, PCM equivalent fibers will always be bundles.

A3.3 Other Structure Counting Criteria

A3.3.1 *Structures* (That Intersect Grid Bars)—Count a structure that intersects a grid bar for two sides of the grid opening only, as illustrated in Fig. A3.4. Record the dimensions of the unobscured portions of the structure and record in the comments section of the report that this structure intersects a grid bar. Do not include structures intersecting either of the other two sides in the count.

A3.3.2 *Fibers* (That Extend Outside the Field of View)—During scanning of a grid opening, count fibers that extend outside of the field of view systematically, so as to avoid double-counting. In general, establish a rule so that fibers extending outside the field of view in only two quadrants are counted. The procedure is illustrated by Fig. A3.5. Measure the length of each such fiber by moving the specimen to locate the other end of the fiber, and then return to the original field of

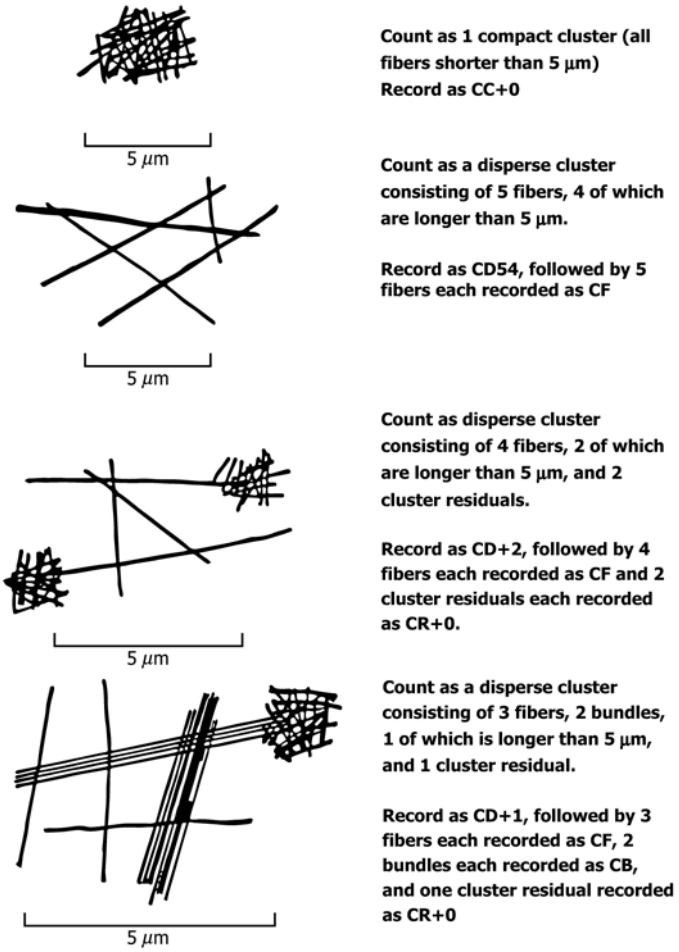


FIG. A3.2 Examples of Recording of Complex Asbestos Clusters

view before continuing to scan the specimen. Do not count fibers without terminations within the field of view.

A3.4 Procedure

A3.4.1 *Introduction*—The morphological codes specified are designed to facilitate computer data processing, and to allow recording of a complete representation of the important features of each asbestos structure. The procedure requires that the microscopist classify each primary fibrous structure into one of the four fundamental categories: fibers, bundles, clusters, and matrices.

A3.4.2 *Fibers*—On the structure counting form, record a fiber as defined in A3.2.1.1 by the designation F. If the fiber is a separately counted part of a cluster or matrix, record the fiber by the designation CF or MF, depending on whether it is a component of a cluster or matrix.

A3.4.3 *Bundles*—On the structure counting form, record a bundle as defined in A3.2.1.2 by the designation B. If the bundle is a separately counted part of a cluster or matrix, record the bundle by the designation CB or MB, depending on whether it is a component of a cluster or matrix.

A3.4.4 *Disperse Clusters (Type D)*—On the structure counting form, record an isolated cluster of Type D as defined in A3.2.1.3 by the designation CD, followed by a two-digit number. The first digit represents the analyst's estimate of the total number of fibers and bundles comprising the structure. The digit shall be from 1 to 9, or designated as + if there are estimated to be more than nine component fibers or bundles. The second digit represents, in the same manner, the total number of fibers and bundles longer than 5 µm contained in the structure. Record the overall dimensions of the cluster in two perpendicular directions representing the maximum dimensions. Separately record in order of decreasing length, up to five component fibers or bundles, using the codes CF (cluster fiber) and CB (cluster bundle). If, after accounting for prominent component fibers and bundles, a group of clustered fibers remains, record this as CR (cluster residual). If the remaining clustered fibers are present as more than one localized group, record more than one cluster residual. Do not record more than five cluster residuals for any cluster. Measure and assign a cluster residual a two-digit number, derived in the same manner as specified for the overall cluster. Optionally, if the

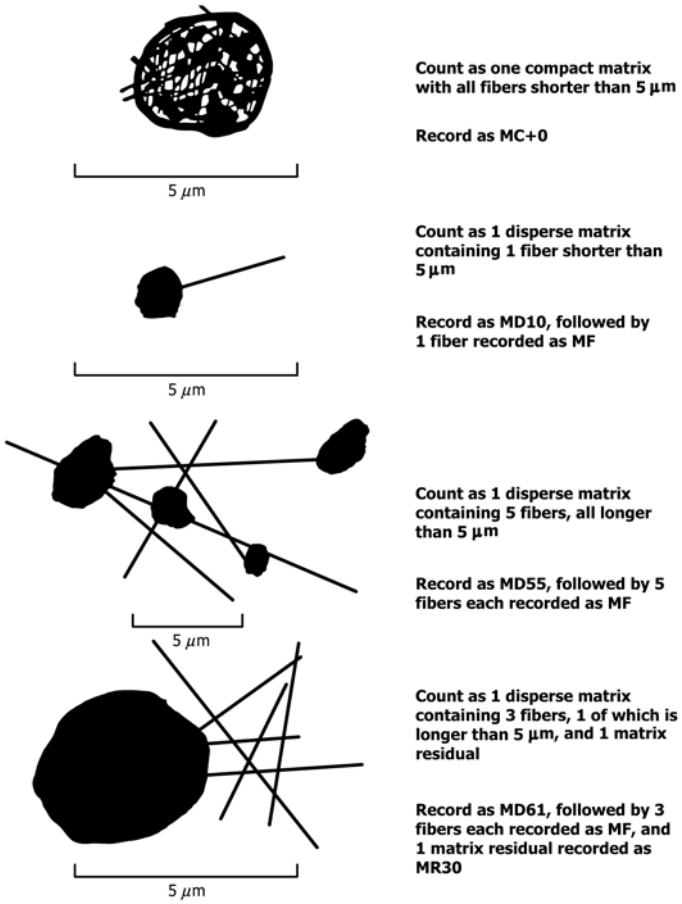


FIG. A3.3 Examples of Recording Complex Asbestos Matrices

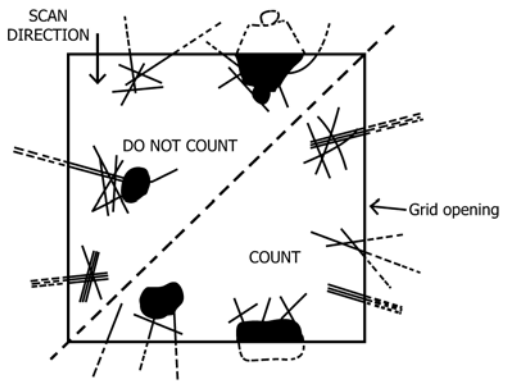


FIG. A3.4 Example of Counting Structures That Intersect Grid Bars

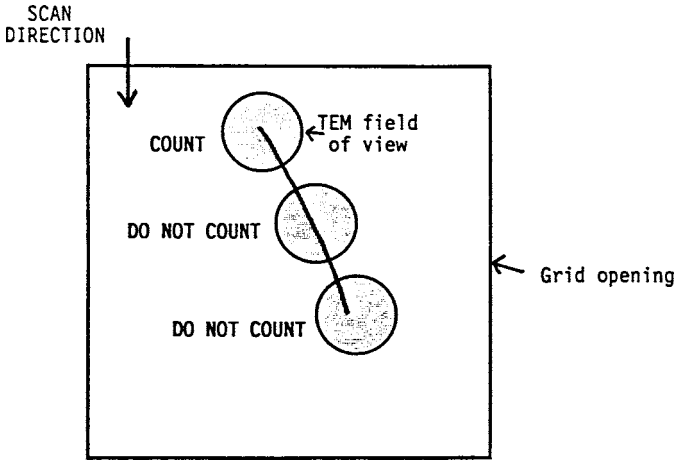


FIG. A3.5 Example of Counting of Fibers That Extend Outside the Field of View

number of component fibers and bundles in either the original cluster or the cluster residual is outside of the range of 1 to 9, note additional information concerning the number of component fibers and bundles in the *comments* column.

A3.4.5 *Compact Clusters (Type C)*—On the structure counting form, record an isolated cluster of Type C as defined in A3.2.1.3 by the designation CC, followed by a two-digit number. Assign the two-digit number describing the numbers of component fibers and bundles in the same manner as for clusters Type D. Record the overall dimensions of the cluster in two perpendicular directions in the same manner as for clusters Type D. By definition, the constituent fibers and bundles of compact clusters cannot be separately measured; therefore, no separate tabulation of component fibers or bundles can be made.

A3.4.6 *Disperse Matrices (Type D)*—On the structure counting form, record an isolated matrix of Type D as defined in A3.2.1.4 by the designation MD, followed by a two-digit

number. Assign the two digit number in the same manner as for clusters Type D. Record the overall dimensions of the matrix in two perpendicular directions in the same manner as for clusters Type D. Record separately, in order of decreasing length, up to five component fibers or bundles using the codes MF (matrix fiber) and MB (matrix bundle). If, after accounting for prominent component fibers and bundles, matrix material containing asbestos fibers remains, record it as MR (matrix residual). If the remaining matrix fibers are present as more than one localized group, it may be necessary to record more than one matrix residual. Do not record more than five matrix residuals for any matrix. Measure and assign matrix residual a two-digit number, derived in the same manner as specified for the overall matrix. Optionally, if the number of component fibers and bundles in either the original matrix or the matrix residual is outside of the range of 1 through 9, note additional information concerning the number of component fibers and bundles in the *comments* column.

A3.4.7 *Compact Matrices (Type C)*—Record on the structure counting form an isolated matrix of Type C as defined in A3.2.1.4 by the designation MC, followed by a two-digit number. Assign the two-digit number in the same manner as for clusters Type D. Record the overall dimensions of the matrix in two perpendicular directions in the same manner as for clusters Type D. By definition, the constituent fibers and bundles of compact matrices cannot be separately measured; therefore, no separate tabulation of component fibers or bundles can be made.

A3.4.8 *Procedure for Recording of Partially Obscured Fibers and Bundles*—Use the proportion of the length of a fiber or bundle that is obscured by other particle as the basis for determining whether a fiber or bundle is to be recorded as a separate component or is to be considered as a part of a matrix Type C or part of a matrix residual. If the obscured length could not possibly be more than one third of the total length, consider the fiber or bundle a prominent feature to be separately recorded. The assigned length for each such partially obscured fiber or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Include fibers or bundles that appear to cross the matrix, and for which both ends can be located approximately, in the maximum of five and record them according to the counting criteria as separate fibers or bundles. If the obscured length could be more than one third of the total length, consider the fiber or bundle as a part of a compact matrix Type C or part of a matrix residual.

A3.5 Special Considerations for Counting of PCM Equivalent Structures

A3.5.1 Use 3:1 as the minimum aspect ratio for counting of PCM equivalent structures. This aspect ratio definition is required to achieve comparability of the results for this size range of structure with historical optical measurements; however, use of this aspect ratio definition does not significantly affect the ability to interpret the whole fiber size distribution in terms of a minimum 5:1 aspect ratio. Some applications may require that a count be made of PCM equivalent structures only. The coding system permits discrimination between PCM equivalent structures that contain fibers and bundles longer than 5 µm and those that do not.

A3.5.2 In general, clusters and matrices will yield fewer components as the minimum dimensions specified for countable fibers are increased. Thus it may be found that a particular structure yields a higher number of component fibers and bundles in a count for all fiber sizes than it does at a reduced magnification when only fibers and bundles longer than 5 µm are being counted. However, the requirement that component fibers and bundles be recorded in decreasing length order ensures that the data are consistent for a particular structure, regardless of the size category of fibers being counted and the magnification in use.

A4. FIBER IDENTIFICATION PROCEDURE

A4.1 Introduction

A4.1.1 Select the criteria used for identification of asbestos fibers depending on the intended use of the measurements. In some circumstances, there can be a requirement that fibers shall be unequivocally identified as a specific mineral species. In other circumstances, there can be sufficient knowledge about the sample that rigorous identification of each fiber need not be carried out. The time required to perform the analysis, and therefore the cost of analysis, can vary widely depending on the identification criteria that are considered to be sufficiently definitive. Specify the combination of criteria considered definitive for identification of fibers in a particular analysis before the analysis is made, and refer to this combination of criteria as the level of analysis. Various factors related to instrumental limitations and the character of the sample may prevent satisfaction of all of the specified fiber identification criteria for a particular fiber. Therefore, prepare a record of the identification criteria that were satisfied for each suspected asbestos fiber included in the analysis. For example, if both ED and EDXA were specified to be attempted for definitive identification of each fiber, fibers with chrysotile morphology that, for some reason, do not give an ED pattern but do yield an EDXA spectrum corresponding to chrysotile are categorized in a way that conveys the level of confidence to be placed in the identification.

A4.2 ED and EDXA Techniques

A4.2.1 *General:*

A4.2.1.1 Initially, classify fibers into two categories on the basis of morphology: those fibers with tubular morphology, and those fibers without tubular morphology. Conduct further analysis of each fiber using ED and EDXA methods. Use the following procedures when fibers are examined by ED and EDXA.

A4.2.1.2 The crystal structures of some mineral fibers, such as chrysotile, are easily damaged by the high current densities required for EDXA examination. Therefore, complete investigation of these sensitive fibers by ED before attempts are made to obtain EDXA spectra from the fibers. When more stable fibers, such as the amphiboles, are examined, EDXA and ED may be used in either order.

A4.2.2 *ED Techniques:*

A4.2.2.1 The ED technique can be either qualitative or quantitative. Qualitative ED consists of visual examination, without detailed measurement, of the general characteristics of the ED pattern obtained on the TEM viewing screen from a randomly oriented fiber. ED patterns obtained from fibers with cylindrical symmetry, such as chrysotile, do not change when the fibers are tilted about their axes, and patterns from randomly-oriented fibers of these minerals can be interpreted quantitatively. For fibers that do not have cylindrical symmetry, only those ED patterns obtained when the fiber is oriented with a principal crystallographic axis closely parallel with the incident electron beam direction can be interpreted quantitatively. Refer to this type of ED pattern as a zone-axis ED pattern. To interpret a zone-axis ED pattern quantitatively, record it photographically and check its consistency with known mineral structures. Compare measurements of the zone-axis ED pattern with corresponding data calculated from known mineral structures by use of a computer program. The zone-axis ED pattern obtained by examination of a fiber in a particular orientation can be insufficiently specific to permit unequivocal identification of the mineral fiber, but it is often possible to tilt the fiber to another angle and to record a different ED pattern corresponding to another zone-axis. Also check the angle between the two zone-axes for consistency with the structure of a suspected mineral.

A4.2.2.2 For visual examination of the ED pattern, adjust the camera length of the TEM to a low value of approximately 250 mm and then view the ED pattern through the binoculars. However, the pattern is distorted by the tilt angle of the viewing screen. Use a camera length of at least 2 m when recording the ED pattern, if accurate measurement of the pattern is to be possible. It is necessary to properly adjust the sample height to the eucentric point when obtaining an ED pattern to be evaluated visually or to be recorded, and to focus the image in the plane of the selected area aperture. Otherwise there may be some components of the ED pattern that do not originate from the selected area. In general, use the smallest available ED aperture.

A4.2.2.3 Use an internal calibration standard for accurate measurements of the ED pattern. Apply a thin coating of gold, or other suitable calibration material, to the underside of the TEM specimen. Apply this coating either by vacuum evaporation or, more conveniently, by sputtering. The polycrystalline gold film yields diffraction rings on every ED pattern and these rings provide the required calibration information.

A4.2.2.4 To form an ED pattern, move the image of the fiber to the center of the viewing screen, adjust the height of the specimen to the eucentric position, and insert a suitable selected area aperture into the electron beam so that the fiber, or a portion of it, occupies a large proportion of the illuminated area. Adjust the size of the aperture and the portion of the fiber such that particles other than the one to be examined are excluded from the selected area. Observe the ED pattern through the binoculars. During the observation, adjust the objective lens current to the point where the most complete ED pattern is obtained. If an incomplete ED pattern is still obtained, move the particle around within the selected area to attempt to optimize the ED pattern or to eliminate possible interferences from neighboring particles.

A4.2.2.5 If a zone-axis ED analysis is to be attempted on the fiber, mount the sample in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fiber image indicates that the fiber is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fiber is at the eucentric position. Tilt the fiber until an ED appears that is a symmetrical, two-dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. Observe the manner in which the intensities of the spots vary during tilting of the fiber to obtain zone-axis conditions. If weak reflections occur at some points on a matrix of strong reflections, the possibility of twinning or multiple diffraction exists. Exercise caution in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the papers by J. A. Gard (**9**), P. B. Hirsch, et al. (**10**), and H. R. Wenk (**11**). Not all zone-axis patterns that can be obtained are definitive. Record only those that have closely-spaced reflections corresponding to low indices in at least one direction. Patterns in which all d-spacings are less than about 0.3 nm are not definitive. A useful guideline is that the lowest angle reflections be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

A4.2.2.6 Select five spots, closest to the center spot, along two intersecting lines of the zone-axis pattern for measurement, as illustrated in Fig. A4.1. The distances of these spots from the center spot and the four angles shown provide the required data for analysis. Since the center spot is usually very overexposed, it does not provide a well defined origin for these measurements. Therefore, obtain the required distances by measuring between pairs of spots symmetrically disposed about the center spot, preferably separated by several repeat distances. Measure the distances with a precision of better than 0.3 mm, and the angles to a precision of better than 2.5°. Also measure the diameter of the first or second ring of the calibration pattern (111 and 200) with a precision of better than 0.3 mm.

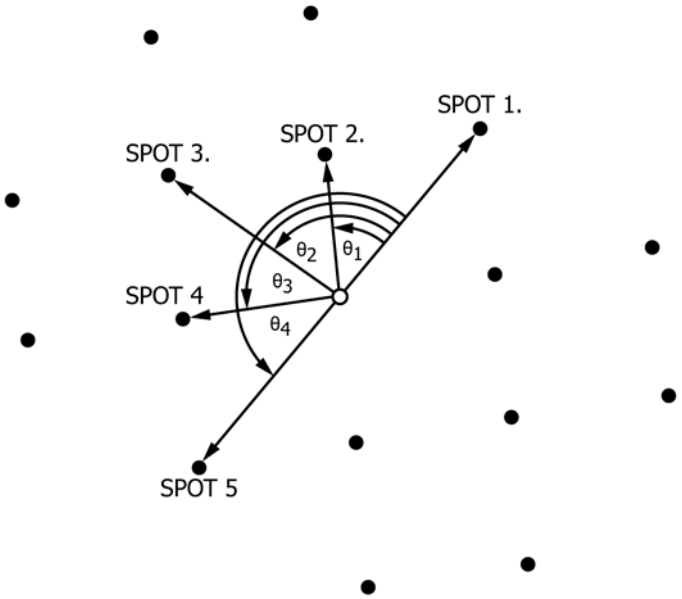


FIG. A4.1 Example of Measurement of Zone-Axis ED Patterns

A4.2.2.7 Using gold as the calibration material, the radius-based camera constant is given by:

$\lambda \times L = 0.11774 \times D$ mm.nm (first ring), and

$\lambda \times L = 0.10197 \times D$ mm.nm (second ring).

A4.2.3 *EDXA Measurements:*

A4.2.3.1 Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, record the X-ray peaks originating from the elements in the fiber. For quantitative interpretation, obtain the net peak areas, after background subtraction, for the X-ray peaks originating from the elements in the fiber. This test method provides quantitative interpretation for those minerals that contain silicon.

A4.2.3.2 To obtain an EDXA spectrum, move the image of the fiber to the center of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fiber. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector and, in some instruments, to use Scanning Transmission Electron Microscopy (STEM) mode of operation.

A4.2.3.3 The time for acquisition of a suitable spectrum varies with the fiber diameter, and also with instrumental factors. For quantitative interpretation, ensure that the spectra have a statistically valid number of counts in each peak. Analyses of small-diameter fibers that contain sodium are the most critical since it is in the low-energy range that the X-ray detector is least sensitive. Accordingly, acquire a spectrum for a sufficiently long period that the presence of sodium can be detected in such fibers. It has been found that satisfactory quantitative analyses can be obtained if acquisition is continued until the background subtracted silicon *Kα* peak integral exceeds 10 000 counts. Manipulate the spectrum to subtract the background and to obtain the net areas of the elemental peaks.

A4.2.3.4 After quantitative EDXA classification of some fibers by computer analysis of the net peak areas, it may be possible to classify further fibers in the same sample on the basis of comparison of spectra at the instrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

A4.3 Interpretation of Fiber Analysis Data

A4.3.1 *Chrysotile*—The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few other minerals have similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the pattern correspond to those from reference chrysotile. However, depending on the past history of the fiber, and on a number of other factors, the crystal structure of a particular fiber may be damaged, and it may not yield an ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

A4.3.2 *Amphiboles:*

A4.3.2.1 Since the fiber identification procedure for asbestos fibers other than chrysotile can be involved and time-consuming, computer programs such as that developed by Rhoades (**12**) are recommended for interpretation of zone-axis ED patterns. The published literature contains composition and crystallographic data for all of the fibrous minerals likely to be encountered in TEM analysis of air samples. Compare the compositional and structural data from the unknown fiber with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fiber identified by quantitative EDXA and two zone axis ED patterns.

A4.3.2.2 Initially clarify suspected amphibole fibers on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, compile a list of minerals that are consistent in composition with that measured for the unknown fiber. Obtain the first zone axis ED pattern, according to the instructions in A4.2.2.

A4.3.2.3 It is possible to specify a particular zone-axis pattern for identification of amphibole since a few patterns are often considered to be characteristic. Unfortunately, for a fiber with random orientation on a TEM grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two pre-selected zone-axes. The most practical approach has been adopted, which is to accept those low index patterns that are easily obtained, and then to test their consistency with the structures of the minerals already preselected on the basis of the EDXA data. Test even the structures of nonamphibole minerals in this preselected list against the zone-axis data obtained for the unknown fiber since nonamphibole minerals in some orientations may yield similar patterns consistent with amphibole structures.

A4.3.2.4 Include in the zone-axis ED interpretation all minerals previously selected from the mineral data file as being

chemically compatible with the EDXA data. This procedure will usually shorten the list of minerals for which solutions have been found. Then process a second set of zone-axis data from another pattern obtained on the same fiber, either as further confirmation of the identification or to attempt elimination of an ambiguity. In addition, check the angle measured between the orientations of the two zone-axes for consistency with the structures of the minerals. Exercise caution in rationalizing the inter-zone axis angle since, if the fiber contains c-axis twinning, the two zone-axis ED patterns may originate from the separate twin crystals. In practice, normally apply the full identification procedure to very few fibers, unless for a particular reason precise identification of all fibers is required.

TABLE A4.2 Classification of Fibers Without Tubular Morphology

UF	-	Unidentified Fibers
AD	-	Amphibole by random orientation ED (shows layer pattern of 0.53 nm spacing)
AX	-	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	-	Amphibole by random orientation ED and qualitative EDXA
AQ	-	Amphibole by Quantitative EDXA
AZ	-	Amphibole by one Zone Axis ED pattern
ADQ	-	Amphibole by random orientation ED and Quantitative EDXA
AZQ	-	Amphibole by one Zone Axis ED pattern and Quantitative EDXA
AZZ	-	Amphibole by two Zone Axis ED patterns with consistent interaxial angle
AZZQ	-	Amphibole by two Zone Axis ED patterns, with consistent interaxial angle, and Quantitative EDXA
NAM	-	Non asbestos Mineral

A4.4 Fiber Classification Categories

A4.4.1 It is not always possible to proceed to a definitive identification of a fiber, which may be due to instrumental limitations or to the actual nature of the fiber. In many analyses a definitive identification of each fiber may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. Take into account for the analytical procedure both instrumental limitations and varied analytical requirements. Accordingly, use a system for fiber classification to permit accurate recording of data. The classifications are shown in Table A4.1 and Table A4.2, and are directed towards identification of chrysotile and amphibole, respectively. Report fibers in the categories.

A4.4.2 The general principle to be followed in this analytical procedure is first to define the most specific fiber classification that is to be attempted or the level of analysis to be conducted. Then, for each fiber examined, record the classification that is actually achieved. Depending on the intended use of the results, establish the criteria for acceptance of fibers as *identified* at any time after completion of the analysis.

A4.4.3 In an unknown sample, confirm chrysotile only if a recorded, calibrated ED pattern from one fiber in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Confirm amphibole only by obtaining recorded data that yield exclusively amphibole solutions for fibers classified in the AZQ, AZZ, or AZZQ categories.

A4.4.4 *Procedure for Classification of Fibers with Tubular Morphology, Suspected to be Chrysotile:*

A4.4.4.1 Occasionally, fibers are encountered that have tubular morphology similar to that of chrysotile, but that cannot be characterized further either by ED or EDXA. They may be non-crystalline, in which case ED techniques are not useful, or they may be in a position on the grid that does not permit an EDXA spectrum to be obtained. Alternatively, the fiber may be of organic origin, but the morphology and composition may not be sufficiently definitive that it can be disregarded. Accordingly, there is a requirement to record each fiber and to specify how confidently each fiber can be identified. Classification of fibers will meet with various degrees of success. Fig. A4.2 shows the classification procedure to be used for fibers that display any tubular morphology. The chart is self-explanatory, and every fiber is either rejected as a nonasbestos mineral (NAM), or classified in some way that by some later criterion could still contribute to the chrysotile fiber count.

A4.4.4.2 Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fiber by ED and EDXA methods according to Fig. A4.2. Where the morphology is more definitive, it may be possible to classify the fiber as having chrysotile morphology (CM).

A4.4.4.3 For classification as CM, the morphological characteristics required are:

(a) The individual fibrils shall have high aspect ratios exceeding 5:1 and be about 30 to 40 nm in diameter;

(b) The electron scattering power of the fiber at 60 to 100 kV accelerating potential shall be sufficiently low for internal structure to be visible; and,

(c) There shall be some evidence of internal structure suggesting a tubular appearance similar to that shown by reference UICC chrysotile, which may degrade in the electron beam.

A4.4.4.4 Examine every fiber having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those that give diffraction patterns with the precise characteristics shown in Fig. A4.3. The relevant features in this pattern for identification of chrysotile are as follows:

(a) Examine the (002) reflections to determine that they correspond closely to a spacing of 0.73 nm;

(b) Ensure the layer line repeat distance corresponds to 0.53 nm; and,

(c) Ensure that the (110) and (130) reflections are streaked

A4.4.4.5 Using the millimeter calibrations on the TEM viewing screen, these observations can readily be made at the instrument. If documentary proof of fiber identification is

TABLE A4.1 Classification of Fibers with Tubular Morphology

TM	-	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	-	Characteristic Chrysotile Morphology
CD	-	Chrysotile ED pattern
CQ	-	Chrysotile composition by Quantitative EDXA
CMQ	-	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	-	Chrysotile ED pattern and composition by Quantitative EDXA
NAM	-	Non-Asbestos Mineral

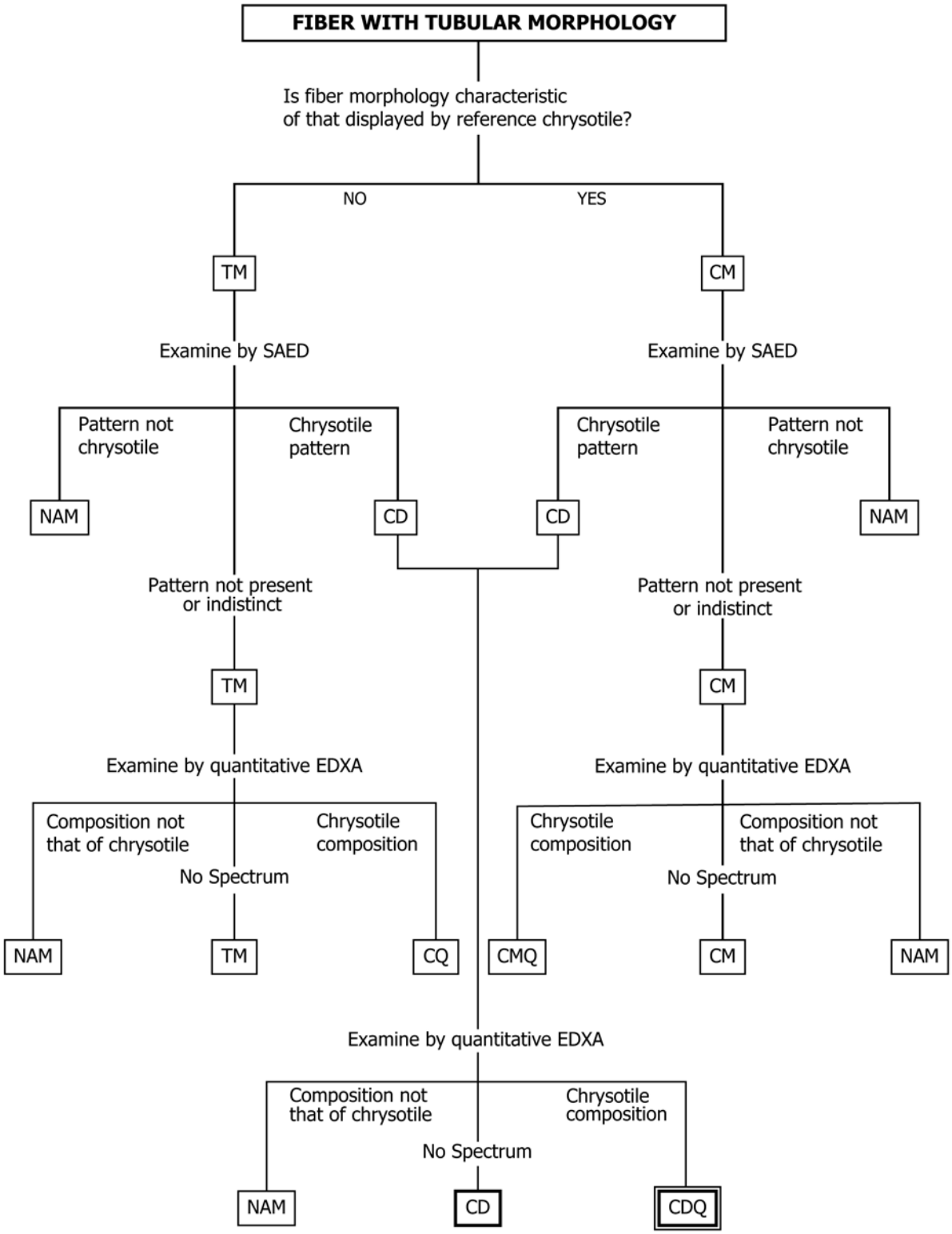


FIG. A4.2 Classification Chart for Fiber with Tubular Morphology

required, an internal calibration standard shall be used to provide accurate measurements of the ED pattern. A thin coating of gold or another suitable calibration material shall be applied to the underside of the TEM specimen. This coating may be applied either by vacuum evaporation or by sputtering. The polycrystalline gold film yields diffraction rings on every ED pattern and these rings provide the required calibration information. The proportion of fibers that can be successfully identified as chrysotile by ED is variable, and to some extent dependent on both the instrument and the procedures of the

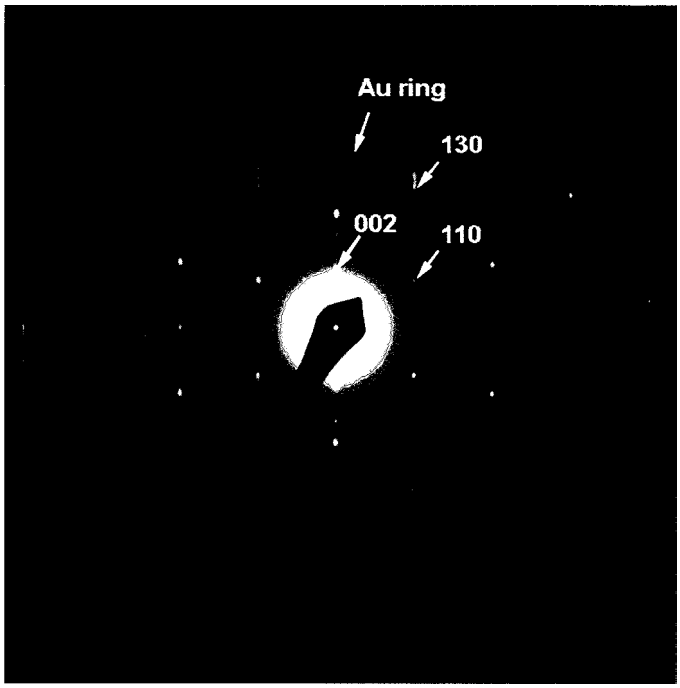


FIG. A4.3 Chrysotile ED Pattern Showing (002) Reflections and Streaked (110) and (130) Reflections

operator. The fibers that fail to yield an identifiable ED pattern will remain in the TM or CM categories unless they are examined by EDXA.

A4.4.4.6 In the EDXA analysis of chrysotile, there are only two elements that are relevant. For fiber classification, the EDXA analysis shall be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their areas in the appropriate ratio, and with only minor peaks from other elements, classify the fiber as chrysotile by quantitative EDXA in the categories CQ, CMQ, or CDQ, as appropriate.

A4.4.5 *Procedure for Classification of Fibers Without Tubular Morphology, Suspected to be Amphibole:*

A4.4.5.1 Consider as a suspected amphibole fiber every particle without tubular morphology and that is obviously not of biological origin, with an aspect ratio of 5:1 or greater, and having parallel or stepped sides. Further examination of the fiber by ED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fiber and on a number of instrumental limitations. It will not be possible to identify every fiber completely, even if time and cost are of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone-axis ED patterns, a very time-consuming procedure.

A4.4.5.2 Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone-axis ED work to a minimum of one fiber representative of each compositional class reported. In some samples, it may be necessary to identify more fibers by the zone-axis technique. When analyzing samples from well-characterized sources, the cost of identification by zone-axis methods may not be justified.

A4.4.5.3 The 0.53 nm layer spacing of the random orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of c-axis twinning in many fibers leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fiber aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fiber is still ambiguous since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fiber may be some other mineral species.

A4.4.5.4 Fig. A4.4 shows the fiber classification chart to be used for suspected amphibole fibers. This chart shows all the classification paths possible in analysis of a suspected amphibole fiber when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fiber by random orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fiber classification assigned will be defined either by successful analysis at the maximum required level or by the instrumental limitations. Note any instrumental limitations that affect the quality of the results. Record the maximum classification achieved for each fiber on the counting sheet in the appropriate column. The various classification categories can be combined later in any desired way for calculation of the fiber concentration. The complete record of the results obtained when attempting to identify each fiber can also be used to reassess the data, if necessary.

A4.4.5.5 In the unknown sample, zone-axis analysis will be required if the presence of amphibole is to be unequivocally confirmed. For this level of analysis, attempt to raise the classification of every suspected amphibole fiber to the ADQ category by inspection of the random orientation ED pattern and the EDXA spectrum. In addition, examine at least one fiber from each type of suspected amphibole found by zone-axis methods to confirm its identification. In most cases, because information exists about possible sources of asbestos in close proximity to the air sampling location, some degree of ambiguity of identification can be accepted. Lower levels of analysis can therefore be accepted for these situations.

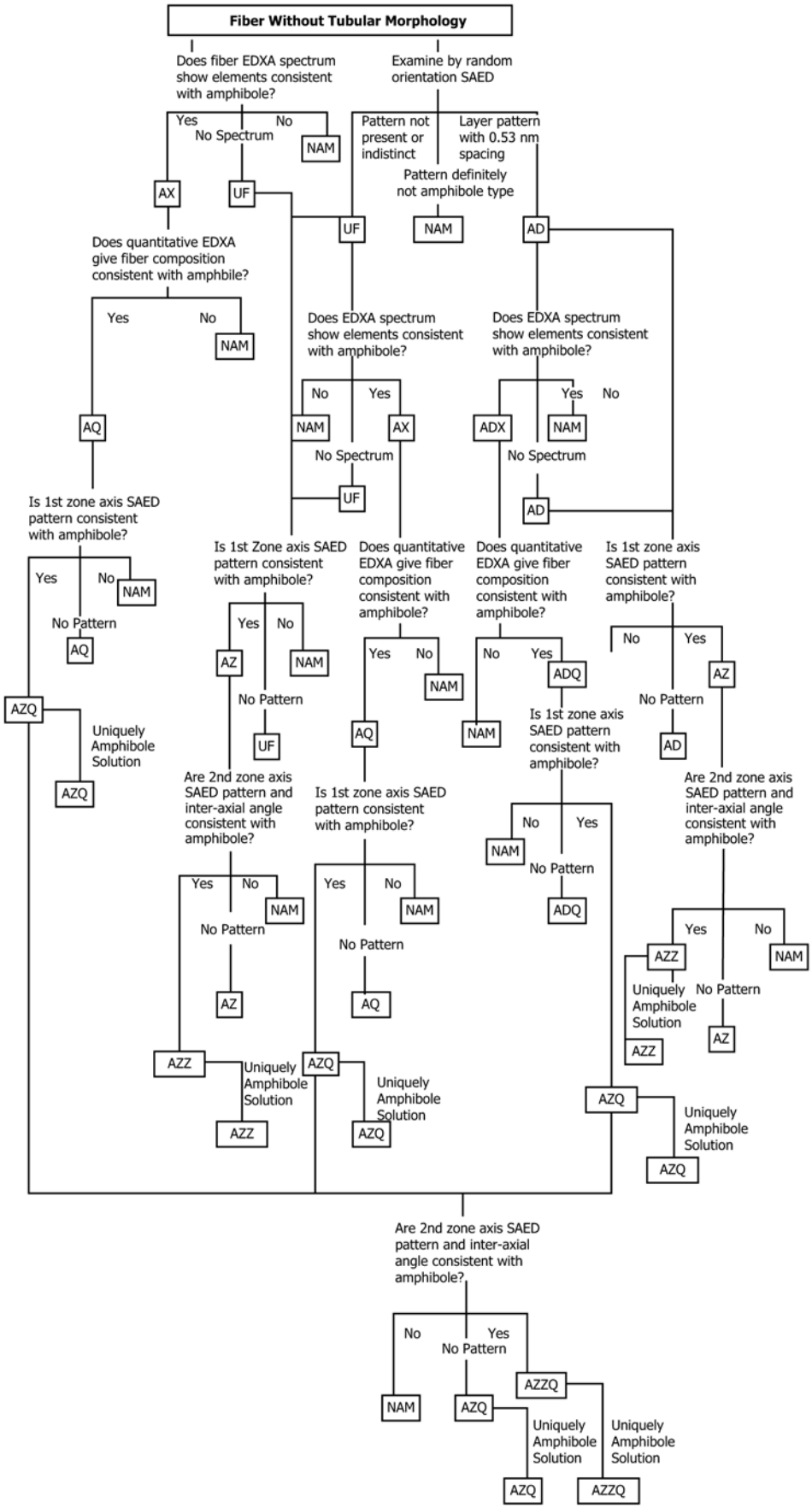


FIG. A4.4 Classification Chart for Fiber Without Tubular Morphology

A5. DETERMINATION OF THE CONCENTRATIONS OF ASBESTOS FIBERS AND BUNDLES LONGER THAN 5 µm, AND PCM EQUIVALENT ASBESTOS FIBERS

A5.1 To provide increased statistical precision and improved analytical sensitivity for those asbestos fibers and bundles longer than 5 µm, it may be decided to perform additional fiber counting at a lower magnification, taking account only of fibers and bundles within this dimensional range. Specify the result as "asbestos fibers and bundles longer than 5 µm." For this examination, use a magnification of approximately 10 000, and continue to assign a morphological code to each structure according to the procedures in Annex A3. Record fibers and bundles only if their lengths exceed 5 µm. Record cluster and matrix components only if their lengths exceed 5 µm.

A5.2 It may also be decided to provide increased statistical precision and improved analytical sensitivity for fibrous structures longer than 5 µm which have diameters between 0.2 and 3.0 µm, which have historically been the basis of risk estimation in the occupational environment (PCM equivalent asbestos fibers). Use a magnification of approximately 5000 for this extended fiber count. Specify the result as "PCM equivalent asbestos fibers."

A5.3 Continue the extended sample examination until 100 asbestos structures have been counted, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity calculated according to Table 1. Divide the examined grid openings approximately equally between a minimum of two specimen grids.

A5.3.1 The specimen area corresponding to the area of filter examined in the PCM fiber counting methods is 0.785 mm^2, and is equivalent to approximately 100 grid openings of a 200 mesh grid.

A5.4 The minimum aspect ratio for definition of a fiber in PCM fiber counting methods and in some standards is 3:1. Use of a 3:1 aspect ratio is permitted in this test method, if this aspect ratio is specified in the test report.

A5.5 Include in the test reports of the items specified in Section 13.

A6. CALCULATION OF RESULTS

A6.1 Introduction

A6.1.1 The calculations described below lead to estimates of airborne concentrations of asbestos in units of structures/L for single samples and the precision associated with those estimates. Precision is most meaningfully expressed as a confidence interval about the estimate. The discussion below indicates how to calculate a 95 % confidence interval for the true mean concentration for a single sample.

A6.2 Test for Poisson Distribution of Primary Fibrous Structures on TEM Grids.

A6.2.1 Structure counts on filters are expected to follow a Poisson probability distribution, and therefore, confidence intervals are determined using the Poisson distribution. The principal characteristic of the Poisson distribution is uniformity in the arrangement of structures on the filter. Specifically, the number of structures expected in a grid opening is proportional to the area of the grid opening. If the distribution of structures on the filter is not uniform, but structures are found in clumps, the Poisson distribution would not be applicable. As a consequence, a confidence interval calculated using the Poisson distribution would be narrower than the correct confidence interval. Stated another way, under these circumstances, the precision of the estimate would be overstated. To test for the correctness of the Poisson distribution, proceed as follows:

A6.2.2 Calculate the number of structures expected in each of the k grid openings inspected as np_i, where $p_i = A_i/A$, $A = \sum A_i$ is the sum of the areas of the k grid openings inspected, n is the total number of structures counted in the sample, and A_i = grid opening area.

A6.2.3 Calculate a chi-squared (X^2) value for the distribution of structures in grid openings as:

$$X^2 = \sum_{i=1}^{i=k} \frac{(n_i - n\,p_i)^2}{n\,p_i} \qquad \text{(A6.1)}$$

where the sum is over the k grid openings that were inspected.

A6.2.4 Determine the p-value of the X^2 calculated in A6.2.3 by referring to a table of percentiles for the X^2 probability distribution with (k-1) degrees of freedom. If the p-value is less than 0.001 (0.1 %), the distribution of structures on the filter lacks uniformity and the Poisson distribution would not apply. Under these circumstances, the width of a confidence interval based on the Poisson distribution would be understated, giving a false sense of precision. To obtain a proper confidence interval consider: (i) investigating application of the Negative

Binomial distribution; (ii) inspecting additional grid openings and applying the method described in A6.4.3.3.

A6.3 Calculation of the Analytical Sensitivity

A6.3.1 Calculate the analytical sensitivity S, in structures/L, using the formula:

$$S = A_f/(K \times A_g \times V) \quad \text{(A6.2)}$$

where:

S = analytical sensitivity in structures/L,
A_f = active area of sample collection filter in mm^2,
K = number of grid openings examined,
A_g = mean area of TEM specimen grid opening in mm^2, and
V = volume of air sampled in L.

A6.4 Calculation of the Mean and Confidence Interval of the Structure Concentration

A6.4.1 *Introduction*—The following demonstrate the methods to determine the mean grid opening structure count for the population on the basis of a small sample, and the interval about the sample mean, which, with 95 % confidence, contains the population mean for a structure count made according to this test method of a number of grid openings sampled from a population of grid openings.

A6.4.2 *Calculation of the Mean Structure Concentration*—Determine the mean structure concentration, C, in structures/L

$$C = S \times n \quad \text{(A6.3)}$$

where:

S = analytical sensitivity in structures/L, and
n = total number of structures found on all grid openings examined.

A6.4.3 *Calculation of Confidence Intervals:*

A6.4.3.1 If the structure distribution on the filter is accepted as Poisson by the procedure in A6.2, a 95 % confidence interval for a sample result may be calculated using Table A6.1. Table A6.1 provides lower and upper 95 % confidence limits for the true mean structure count. A95 % confidence interval for the true mean structure concentration is obtained by multiplying the lower and upper limits from Table A6.1 by the analytical sensitivity of the measurement.

A6.4.3.2 Table A6.1 may be used to construct two-sided confidence intervals for the Poisson distribution corresponding

TABLE A6.1 Upper and Lower Limits of the Poissonian 95 % Confidence Interval of a Count[A]

Structure Count	Lower	Upper	Structure Count	Lower	Upper	Structure Count	Lower	Upper
0	0	3.689*	41	29.421	55.622	82	65.219	101.79
1	0.025	5.572	42	30.269	56.772	83	66.111	102.90
2	0.242	7.225	43	31.119	57.921	84	67.003	104.00
3	0.619	8.767	44	31.970	59.068	85	67.897	105.11
4	1.090	10.242	45	32.823	60.214	86	68.790	106.21
5	1.624	11.669	46	33.678	61.358	87	69.684	107.32
6	2.202	13.060	47	34.534	62.501	88	70.579	108.42
7	2.814	14.423	48	35.392	63.642	89	71.474	109.53
8	3.454	15.764	49	36.251	64.781	90	72.370	110.63
9	4.115	17.085	50	37.112	65.919	91	73.267	111.73
10	4.795	18.391	51	37.973	67.056	92	74.164	112.83
11	5.491	19.683	52	38.837	68.192	93	75.061	113.94
12	6.201	20.962	53	39.701	69.326	94	75.959	115.04
13	6.922	22.231	54	40.567	70.459	95	76.858	116.14
14	7.654	23.490	55	41.433	71.591	96	77.757	117.24
15	8.396	24.741	56	42.301	72.721	97	78.657	118.34
16	9.146	25.983	57	43.171	73.851	98	79.557	119.44
17	9.904	27.219	58	44.041	74.979	99	80.458	120.53
18	10.668	28.448	59	44.912	76.106	100	81.360	121.66
19	11.440	29.671	60	45.785	77.232	110	90.400	132.61
20	12.217	30.889	61	46.658	78.357	120	99.490	143.52
21	13.000	32.101	62	47.533	79.482	130	108.61	154.39
22	13.788	33.309	63	48.409	80.605	140	117.77	165.23
23	14.581	34.512	64	49.286	81.727	150	126.96	176.04
24	15.378	35.711	65	50.164	82.848	160	136.17	186.83
25	16.178	36.905	66	51.042	83.969	170	145.41	197.59
26	16.983	38.097	67	51.922	85.088	180	154.66	208.33
27	17.793	39.284	68	52.803	86.207	190	163.94	219.05
28	18.606	40.468	69	53.685	87.324	200	173.24	229.75
29	19.422	41.649	70	54.567	88.441	210	182.56	240.43
30	20.241	42.827	71	55.451	89.557	220	191.89	251.10
31	21.063	44.002	72	56.335	90.673	230	201.24	261.75
32	21.888	45.175	73	57.220	91.787	240	210.60	272.39
33	22.715	46.345	74	58.106	92.901	250	219.97	283.01
34	23.545	47.512	75	58.993	94.014	260	229.36	293.62
35	24.378	48.677	76	59.880	95.126	270	238.75	304.23
36	25.213	49.840	77	60.768	96.237	280	248.16	314.82
37	26.050	51.000	78	61.657	97.348	290	257.58	325.39
38	26.890	52.158	79	62.547	98.458	300	267.01	335.96
39	27.732	53.315	80	63.437	99.567	350	314.29	388.68
40	28.575	54.469	81	64.328	100.68	400	361.76	441.21

[A] The one-sided upper 95 % confidence limit for zero structures is 2.99.

to counts from zero up to 470. When the count is zero, however, a one-sided 95 % confidence interval based on the upper confidence limit provides a more realistic range. The 95 % confidence limit in this case would be from 0 structures/L to (2.99 times the analytical sensitivity) structures/L. (Note that 2.99 is the upper 95 % confidence limit for the Poisson distribution when the observed sample count is zero.)

A6.4.3.3 If the structure distribution on the filter is not accepted as Poisson by the procedure in A6.2, consider inspecting additional grid openings (for example, original plus additional equal to at least 30) and applying the Normal (Gaussian) probability distribution to determine a confidence interval.

A6.4.3.4 The calculations for a two-sided 95 % confidence interval based on the Normal distribution is as follows:

(a) Calculate the sample standard deviation as:

$$s^2 = \sum_{i=1}^{i=k} \frac{(n_i - n/k)^2}{(k-1)} \quad \text{(A6.4)}$$

where:

n_i = number of structures on the i'th grid opening,

n = total number of structures found in k grid openings, and

k = number of grid openings inspected.

(b) Calculate the lower and upper 95 % confidence limits for the true mean structure count per grid opening as:

$$n_L = (n/k) - (ts/k^{1/2}) \quad \text{(A6.5)}$$

and

$$n_U = (n/k) + (ts/k^{1/2}) \quad \text{(A6.6)}$$

where:

n_U = upper 95 % confidence limit,

n_L = lower 95 % confidence limit,

n = total number of structures in all grid openings examined,

t = the 97.5 percentile point from Student's "t" with (k-1) degrees of freedom,

s = standard deviation, and

k = number of grid openings.

A6.4.3.5 The lower and upper 95 % confidence limits for Structure/Liter are, respectively, n_L and n_U multiplied by k times the sensitivity for the measurement.

APPENDIXES

(Nonmandatory Information)

X1. CALCULATION OF STRUCTURE LENGTH, WIDTH, AND ASPECT RATIO DISTRIBUTIONS

X1.1 Introduction

X1.1.1 The distributions are all approximate to logarithmic-normal, so the size range intervals for calculation of the distribution are spaced logarithmically. The other characteristics required for the choice of size intervals are that they allow for a sufficient number of size classes, while still retaining a statistically-valid number of structures in each class. Interpretation is also facilitated if each size class repeats at decade intervals, and if 5 µm is a size class boundary. A ratio from one class to the next of 1.468 satisfies all of these requirements and this value shall be used. The distributions, being approximately logarithmic-normal, when presented graphically, are plotted using a logarithmic ordinate scale and a Gaussian abscissa.

X1.2 Calculation of Structure Length Cumulative Number Distribution

X1.2.1 This distribution allows the fraction of the total number of structures either shorter or longer than a given length to be determined. It is determined as follows:

$$C(P)_k = \frac{\sum_{i=l}^{i=k} n_i}{\sum_{i=l}^{i=P} n_i} \times 100 \quad \text{(X1.1)}$$

where:

$C(P)_k$ = cumulative number percentage of structures that have lengths less than the upper bound of the k'th class,

n_i = number of structures in the i'th length class, and

P = total number of length classes.

X1.3 Calculation of Structure Width Cumulative Number Distribution

X1.3.1 This distribution allows the fraction of the total number of structures either narrower or wider than a given width to be determined. It is determined in a similar way to that used in X1.2, but using the structure widths.

X1.4 Calculation of Structure Aspect Ratio Cumulative Number Distribution

X1.4.1 This distribution allows the fraction of the total number of structures that have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is determined in a way similar to that used in X1.2, but using the structure aspect ratios.

X2. METHODS FOR REMOVAL OF GYPSUM FIBERS

X2.1 It is common to find fibers of calcium sulfate (gypsum) in airborne particulate matter collected in buildings and urban environments, and particularly in samples collected where demolition or construction work is in progress. The fibers are readily released when plasters and cement products are disturbed. In some circumstances, particles of calcite or dolomite collected on an air filter can react with atmospheric sulfur dioxide to form long fibers of gypsum. Gypsum fibers can give rise to high fiber counts by both optical and electron microscopes. The gypsum fibers are often 2 to 6 µm long, with aspect ratios greater than 10:1. Sometimes these fibers appear similar to amphibole asbestos fibers, and in some samples, they can be morphologically very similar to chrysotile. In the TEM the larger fibers have high contrast and at high magnification often exhibit a characteristic mottled appearance that changes under electron beam irradiation. Some gypsum fibers, however, are not easily discriminated from asbestos without examination by EDXA. TEM specimens that contain many such gypsum fibers require an extended examination time in the TEM because it is necessary to examine each of these fibers by EDXA before it can be rejected.

X2.2 It is possible to remove gypsum fibers selectively by water extraction. Prepare a Jaffe washer, or a condensation washer, as described in 6.2.7, but using distilled water as the solvent. Place the TEM specimens which have been prepared previously and examined initially in the TEM, in the washer to allow dissolution of the fibers. If a Jaffe washer is used, the treatment time can be reduced by heating the washer to 90 to 100°C for a few minutes. If a condensation washer is used, the gypsum fibers will be dissolved by treatment for approximately 10 min. The effect of this treatment is to remove the gypsum fibers, leaving carbon replicas that are readily distinguished from asbestos fibers.

X2.2.1 Use this procedure only when examination of the untreated TEM specimen grids shows the gypsum fibers to be isolated from any asbestos fibers present. Losses of asbestos fibers may occur if matrices of gypsum and asbestos are exposed to this procedure.

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