



Designation: D6281 – 09

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 method² 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/mm². 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

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.

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:

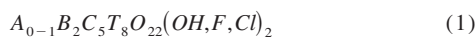
¹ This test method is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.07 on Sampling and Analysis of Asbestos.

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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>.



where:

A = K, Na, Ca,

B = Fe²⁺, Mn, Mg, Ca, Na,

C = Al, Cr, Ti, Fe³⁺, Mg, Fe²⁺, Mn, and

T = Si, Al, Cr, Fe³⁺, 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 Mg₃Si₂O₅(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 Al³⁺ 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 *electron diffraction*—techniques in electron microscopy, including selected area electron diffraction (SAED) and microdiffraction, by which the crystal structure of a specimen is examined.

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

3.2.18 *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.

3.2.19 *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.

3.2.20 *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.

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

3.2.22 *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.

3.2.23 *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.

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

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

3.2.26 *limit of detection*—the calculated airborne asbestos structure concentration in structures/L, equivalent to counting 2.99 asbestos structures in the analysis. The *detection limit* has been set at 2.99 structures counted in any area of any filter

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

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

because of concerns that false positives (counting a structure when none exists) may occur in both blanks and sample filters. Based on the assumption of a Poisson distribution of false positives, the detection limit of 2.99 would protect against a false positive rate as high as 5 % (5 false positive structures per 100 blank filters counted). This level is very conservative, since the actual false positive rate is believed to be 2 % or lower. Thus, many of the samples reported as being below the *detection limit* (less than three structures counted) will actually contain true positives. Note that concentration values are included in the test report, even if they are below the limit of detection.

3.2.27 *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.

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

3.2.29 *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

3.2.30 *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 .

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

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

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

3.2.34 *serpentine*—a group of common rock-forming minerals having the nominal formula: $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$.

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

3.2.36 *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.

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

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

3.3 Symbols:

<i>eV</i>	= electron volt
<i>kV</i>	= kilovolt
<i>L/min</i>	= liters per minute
μg	= micrograms (10^{-6} g)
μm	= micrometer (10^{-6} m)
<i>nm</i>	= nanometer (10^{-9} m)
<i>W</i>	= watt
<i>Pa</i>	= 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 in which asbestos structures are likely to be present. 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

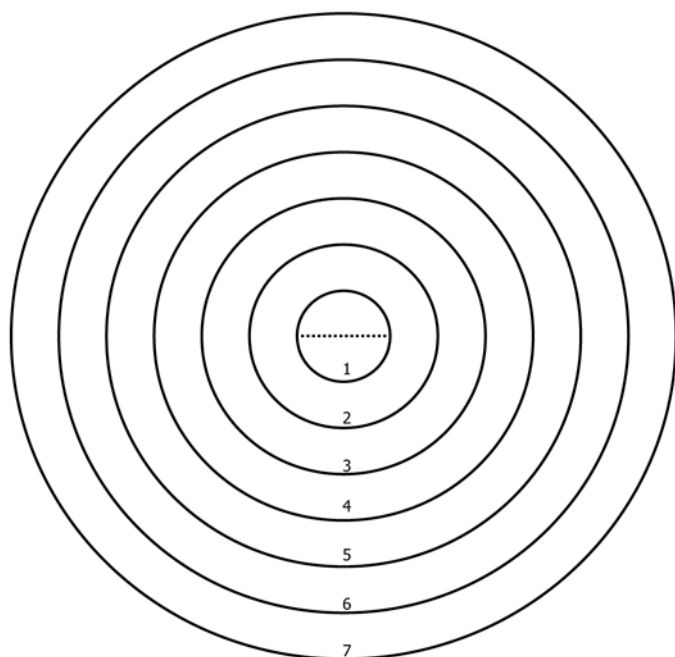


FIG. 1 Example of Calibration Markings on TEM Viewing Screen

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

$$A = 0.7854 \times (D/M + 2000 \times C_s \theta^3)^2 \quad (2)$$

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 $\text{MnK}\alpha$ 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 $\text{NaK}\alpha$ 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.

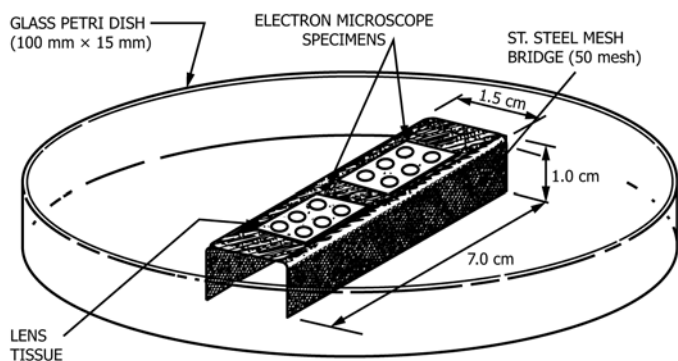
6.2.4 *Plasma Asher*, for preparation of TEM specimens from MCE filters. The plasma asher shall have a radio frequency power rating of 50 W or higher and be provided with a controlled, filtered oxygen flow. Admission of filtered air shall be through a valve to control the speed of air admission so that 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



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)

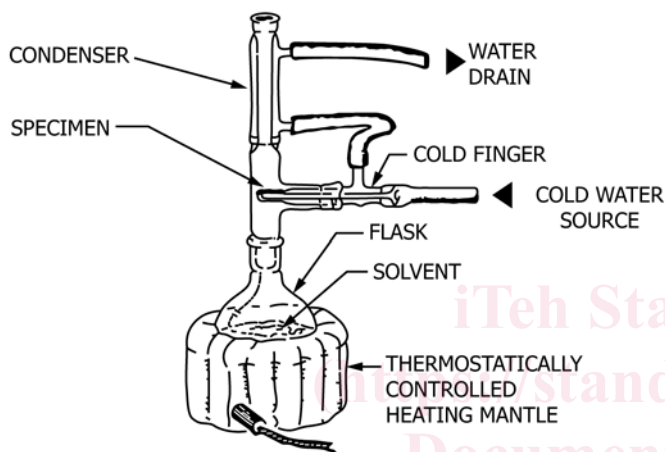


FIG. 3 Design of Condensation Washer

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 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.

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, mm²,

A_g = mean area of TEM specimen grid openings, mm²,

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.

TABLE 1 Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity for a Collection Filter Area of 385 mm² and TEM Grid Openings of 85 μm (0.0072 mm²)

Analytical Sensitivity Structures/L	Volume of Air Sampled, 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

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-pyrrolidone 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:** _____ **mm²**
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		Class	Structure	Length (mm)	Width (mm)	Comments
		Primary	Total					

FIG. 4 Example of Structure Counting Form