Designation: D 6480 – 99

Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy[1]

This standard is issued under the fixed designation D 6480; 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 covers a procedure to identify asbestos in samples wiped from surfaces and to provide an estimate of the concentration of asbestos reported as the number of asbestos structures per unit area of sampled surface. The procedure outlined in this test method employs an indirect sample preparation technique. It is intended to disperse aggregated asbestos into fundamental fibrils, fiber bundles, clusters, or matrices. However, as with all indirect sample preparation techniques, the asbestos observed for quantification may not represent the physical form of the asbestos as sampled. More specifically, the procedure described neither creates nor destroys asbestos, but it may alter the physical form of the mineral fiber aggregates.

1.2 This test method describes the equipment and procedures necessary for wipe sampling of surfaces for levels of asbestos structures. The sample is collected onto a particle-free wipe material (wipe) from the surface of a sampling area that may contain asbestos.

1.2.1 The collection efficiency of this wipe sampling technique is unknown and will vary among substrates. Properties influencing collection efficiency include surface texture, adhesiveness, and other factors.

1.2.2 This test method is generally applicable for an estimate of the concentration of asbestos structures starting from approximately 1000 asbestos structures per square centimetre.

1.3 Asbestos identification by transmission electron microscopy (TEM) is based on morphology, electron diffraction (ED), and energy dispersive X-ray analysis (EDXA).

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

1.4.1 This test method cannot always discriminate between individual fibers of the asbestos and nonasbestos analogues of the same amphibole mineral.

1.4.2 There is no lower limit to the dimensions of asbestos fibers that can be detected. However, in practice, the lower limit to the dimensions of asbestos fibers, that can be detected, is variable and dependent on individual microscopists. Therefore, a minimum length of 0.5 μm has been defined as the shortest fiber to be incorporated in the reported results.

1.5 *This test method 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 test method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres[3]

D 3670 Guide for Determination of Precision and Bias of Methods of Committee D-22[3]

2.2 *Government Standard:*

40 CFR 763, USEPA, Asbestos-Containing Materials in Schools: Final Rule and Notice, Appendix A to Sub-part E[4]

2.3 *U.S. Environmental Protection Agency Standards:*[4]

EPA 600/4-83-043 Analytical Method for the Determination of Asbestos in Water

EPA 747-R-95-001 USEPA, Residential Sampling for Lead: Protocols for Dust and Soil Sampling: Final Report

3. Terminology

3.1 *Definitions*—For definitions of general terms used in this test method, refer to Terminology D 1356.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *amphibole asbestos*—amphibole in an asbestiform habit (**1**).[5]

3.2.2 *analytical sensitivity*—the calculated asbestos structure concentration in asbestos structures/square centimetre, equivalent to counting of one asbestos structure in the analysis calculated using Eq 2.

3.2.3 *asbestos*—a collective term that describes a group of naturally occurring, inorganic, highly fibrous, silicate minerals,

[1] This test method is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis of Atmospheres and is the direct responsibility of Subcommittee D22.07 on Sampling and Analysis of Asbestos.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.03.

[4] Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

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

that are easily separated into long, thin, flexible, strong fibers when crushed or processed (**1-3**).

3.2.3.1 *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 in accordance with nomenclature of the International Mineralogical Association (**3,4**).

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

3.2.4 *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.

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

3.2.6 *bundle*—a structure composed of three or more fibers in a parallel arrangement with the fibers closer than one fiber diameter to each other.

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

3.2.8 *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.

3.2.9 *cluster*—a structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group; groupings of fibers must have more than two points touching.

3.2.10 *d-spacing or inter-planar spacing*—the perpendicular distance between identical adjacent and parallel planes of atoms in a crystal.

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

3.2.12 *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.

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

3.2.14 *fiber*—an elongate particle with parallel or stepped sides. For the purposes of this test method, a fiber is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0.5 μm (see 40 CFR 763).

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

3.2.16 *fibrous mineral*—a mineral composed of parallel, radiating, or interlaced aggregates of fibers from which the fibers are sometimes separable. That is, the crystalline aggregate may be referred to as fibrous even if it is not composed of separable fibers but has that distinct appearance. The term fibrous is used in a general mineralogical way to describe aggregates of grains that crystallize in a needle-like habit and appear to be composed of fibers. Fibrous has a much more general meaning than asbestos. While it is correct that all asbestos minerals are fibrous, not all minerals having fibrous habits are asbestos.

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

3.2.18 *field wipe blank*—a clean, unused, moistened wipe from the same supply that is used for sampling. Field wipes shall be processed in the same manner used to collect field samples with the exception that no surface is wiped. Each wipe designated as a field wipe should be removed from the bulk pack, moistened, and folded in the same manner as the field samples and placed in a sample container labeled as field wipe.

3.2.19 *filter blank*—an unused, unprocessed filter of the type used for liquid filtration.

3.2.20 *filtration blank*—a filter prepared from 250 mL of water.

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

3.2.22 *indirect preparation*—a method in which a sample passes through one or more intermediate steps prior to final filtration. The particles are removed from the original medium and deposited on a second filter prior to analysis.

3.2.23 *limit of detection*—the limit of detection for a measurement by this test method is 2.99 multiplied by the analytical sensitivity for the measurement.

3.2.23.1 *Discussion*—This limit of detection is based on the assumption that the count resulting from potential filter contamination, sample preparation contamination, and other uncontrollable background sources is no greater than 0.05 structures per sample. At this time, however, this subcommittee has no empirical data to confirm this rate.

3.2.24 *matrix*—a structure in which one or more fibers, or fiber bundles that are touching, are attached to, or partially concealed by, a single particle or connected group of nonfibrous particles. The exposed fiber must meet the fiber definition.

3.2.25 *process blank*—an unused wipe (that has not been taken into the field) processed in accordance with the entire preparation and analytical procedure.

3.2.26 *replicate sampling*—one of several identical procedures or samples.

3.2.27 *serpentine*—a group of common rock-forming minerals having the nominal formula: $Mg_3Si_2O_5(OH)_4$. For further information see Ref. (**4**).

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

[6] The nonasbestiform variations of the minerals indicated in 3.2.3.1 have different Chemical Abstract Service (CAS) numbers.

3.2.29 *structure number concentration*—concentration expressed in terms of asbestos structure number per unit of surface area.

3.2.30 *zone-axis*—the crystallographic direction of a crystal that is parallel to the intersecting edges of the crystal faces defining the crystal zone.

3.3 *Symbols*:

eV = electron volt
h = hour
J = joule
kV = kilovolt
min = minute(s)
mL = millilitre (10^{-3} litre)
μL = microlitre (10^{-6} litre)
mm = millimetre (10^{-3} metre)
μm = micrometre (10^{-6} metre)
nm = nanometre (10^{-9} metre)
s = second(s)
W = watt
Pa = pascals

3.4 *Acronyms*:

DMF = dimethyl formamide
ED = electron diffraction
EDXA = energy dispersive X-ray analysis
FWHM = full width, half maximum
HEPA = High Efficiency Particulate Air
MCE = mixed cellulose ester and also refers to pure cellulose nitrate filters
PC = polycarbonate
TEM = transmission electron microscope

4. Summary of Test Method

4.1 Wiping a surface of known area with a wipe material collects a sample. The sample is transferred from the wipe material to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane filter. A section of the membrane filter is prepared and transferred to a TEM grid, using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using ED and EDXA at a magnification from 15 000 - 20 000 X.

5. Significance and Use

5.1 This wipe sampling and indirect analysis test method is used for the general testing of surfaces for asbestos. It is used to assist in the evaluation of surfaces in buildings, such as ceiling tiles, shelving, electrical components, duct work, and so forth. This test method provides an index of the concentration of asbestos structures per unit area sampled as derived from a quantitative measure of the number of asbestos structures detected during analysis.

5.1.1 This test method does not describe procedures or techniques required for the evaluation of the safety or habitability of buildings with asbestos-containing materials, or compliance with federal, state, or local regulations or statutes. It is the user's responsibility to make these determinations.

5.1.2 At present, a single direct relationship between asbestos sampled from a surface and potential human exposure does not exist. Accordingly, the user should consider these data in relationship to other available information (for example, air sampling data) in their evaluation.

5.2 One or more large asbestos-containing particles dispersed during sample preparation may result in large asbestos concentration results in the TEM analyses of that sample. It is, therefore, recommended that multiple replicate independent samples be secured in the same area, and that a minimum of three such samples be analyzed by the entire procedure.

6. Interferences

6.1 The following materials have properties (that is, chemical composition or crystalline structure) that are very similar to asbestos minerals and may interfere with the analysis by causing a false positive to be recorded during the test. Therefore, literature references for these materials shall be maintained in the laboratory for comparison with asbestos minerals so that they are not misidentified as asbestos minerals.

6.1.1 Antigorite,
6.1.2 Fibrous talc,
6.1.3 Halloysite,
6.1.4 Hornblende and other amphiboles,
6.1.5 Palygorskite (attapulgite),
6.1.6 Pyroxenes,
6.1.7 Sepiolite, and
6.1.8 Vermiculite scrolls.

7. Apparatus

7.1 *Equipment and Materials for Sampling*:

7.1.1 *Disposable Wet Towels.*

7.1.2 *Masking Tape.*

7.1.3 *Measuring Tape.*

7.1.4 *Powderless, Rubber Gloves.*

7.1.5 *Sample Container*, clean, sealable, used for transporting the sample to the laboratory.

7.1.6 *Template to Delineate Sampling Area*, a reusable or disposable template of nonparticle-shedding material, such as aluminum, plastic, or nonshedding cardboard. A variety of shapes (for example, square, rectangular) are acceptable. All templates shall have accurately known inside dimensions. Templates should be thin (less than ⅛ in. (3 mm)) and capable of lying flat on a flat surface. Clean reusable template before and after each use with a suitable cleaning method, such as surfactant solution or particle-free disposable wipe.

7.1.7 *Wipe*, particle free, sealed edge, continuous filament cloth sampling medium. Satisfactory brands are available through commercial scientific suppliers. This material is commonly listed under *clean room wiper*. Wipe brands or sources should not contain unacceptable particle or fiber levels. Prior to use, TEM analysis on blank wipe preparations should be performed to determine that background particle and fiber levels will not interfere with preparation and analysis.

7.2 *Equipment and Materials for Preparation*:

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

7.2.2 *Carbon Rod Sharpener*—An instrument used to sharpen carbon rod electrodes.

7.2.3 *Cork Borer*, 7-mm diameter.

7.2.4 *Disposable Tip Micropipettes*, 30 μL.

7.2.5 *Electron Microscope Grids (for example, Cu Au, Ni)*, 200 mesh TEM grids with grid openings of uniform size. Use grids with numerical or alphabetical indexing, or both, of individual grid openings to facilitate the relocation of individual grid openings for quality assurance purposes.

7.2.6 *Filtration Unit*, 25 or 47-mm filter funnel (either glass or disposable). Filter funnel assemblies, either glass or disposable plastic, using a 25 or 47-mm diameter filter.

7.2.7 *Graduated, Disposable Pipettes*, 1, 5, or 10–mL sizes, glass or plastic.

7.2.8 *Grid Box*, for electron microscope grid storage.

7.2.9 *High Efficiency Particulate Air (HEPA) Filtered Negative Flow Hood.*

7.2.10 *Mixed Cellulose Ester (MCE) Membrane Filters*, 25 or 47-mm diameter, ≤ 0.22 and 5-μm pore size.

7.2.11 *pH Paper.*

7.2.12 *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.

7.2.13 *Plastic Petri Dishes*, or similar container to retain filters (50 mm in diameter or larger). These petri dishes may be used as storage containers for archiving filters.

7.2.14 *Polycarbonate (PC) Membrane Filters*, 25 or 47-mm diameter ≤ 0.2-μm pore size.

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

7.2.16 *Side Arm Filter Flask*, 1000 mL.

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

7.2.18 *Specimen Bottle*, wide mouth, sealable, capable of accommodating the wipe and a minimum of approximately 500 mL of distilled water.

7.2.19 *Sputter Coater*, for deposition of gold onto TEM specimens to be used as an internal calibration of ED patterns. Other calibration materials are also acceptable. Experience has shown that a sputter coater allows control of the deposition thickness of the calibration material.

7.2.20 *Solvent Washer* (Jaffe washer) (see EPA 600/4-83-043), 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. 1. Use dimethyl formamide or acetone for dissolving MCE or cellulose nitrate filters. Use either chloroform or 1-methyl-2-pyrrolidone, or a mixture of 20 % 1-2-diaminoethane and 80 % 1-methyl-2-pyrrolidone, or dissolving PC filters. The higher evaporation rates of chloroform and acetone require that a reservoir of 10 to 50 mL of solvent be used, that may need replenishment during the procedure. DMF and 1-methyl-2-pyrrolidone have lower vapor pressures, and 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.

7.2.21 *Ultrasonic Bath*, table top model (100 W).

7.2.22 *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 that will allow a glass microscope slide to be tilted and continuously rotated during the coating procedure is recommended. 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.

7.2.23 *Vacuum Pump*, able to maintain a vacuum of at least 20 kPa.

7.3 *Equipment and Materials for Analysis*:

7.3.1 *Calibration Specimen Grids for EDXA Calibration*—TEM specimen grids prepared from dispersion of calibration minerals required for calibration of the EDXA system: crocidolite (NIST SRM 1866) and chrysotile.

7.3.2 *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. The performance of an individual TEM-EDXA system is dependent on a number of geometrical factors. Therefore, the required performance of the TEM-EDXA system is specified in terms of the measured X-ray obtained from a fiber of small diameter, using a known electron beam diameter. Solid state X-ray detectors are least sensitive in the

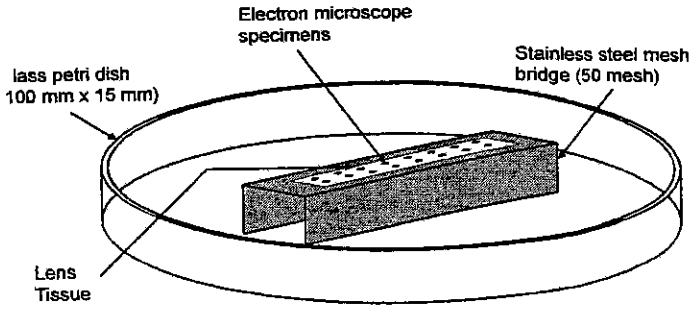

FIG. 1

low energy region; therefore, measurement of sodium in crocidolite shall be the performance criterion. Irradiation of a UICC crocidolite fiber (50 nm or smaller diameter) by an electron probe (250 nm or smaller diameter), the TEM-EDXA system shall yield, under routine analytical conditions, a background-subtracted NaKα integrated peak count rate of more than 1 count per second (cps). The peak/background ratio for this performance test shall exceed 1:0.

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

7.3.3 *Grating Replica*, approximately 2000 parallel lines per mm, used to calibrate the magnification of the TEM.

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

7.3.5 *Transmission Electron Microscope*—A TEM operating at an accelerating potential from 80 to 120 kV, with a resolution better than 1.0 nm, and a magnification range of approximately 300 to 100 000 X shall be used, with the ability to obtain a screen magnification of about 100 000 X, 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 gradation in the form of circles.

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

$$A = 0.7854\left[\frac{D}{M} + 2000C_s\theta^3\right]^2 \quad (1)$$

where:

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

7.3.5.2 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.

7.3.5.3 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.

7.3.5.4 The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fiber, the goniometer shall be of a type that permits tilting of the specimen and acquisition of EDXA spectra without change of specimen holder. If the goniometer does not permit eucentric tilting, gold or other metal film must be evaporated on the sample in order that ED patterns may be accurately calibrated.

7.3.5.5 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.

8. Reagents

8.1 *Reagents for Sample Preparation*:

8.1.1 *1-Methyl-2-pyrrolidone*, analytical grade.

8.1.2 *1-2-diaminoethane*, analytical grade.

8.1.3 *Acetone*, analytical grade.

8.1.4 *Alcohol*, ethanol, 2-propanol, or methanol.

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

8.1.6 *Dimethyl Formamide*, analytical grade.

8.1.7 *Glacial Acetic Acid*, analytical grade.

8.1.8 *Purity of Water*—References to water shall be understood to mean reagent water as defined by Type I of Specification D 1193, or by distilled or deionized water filtered through a membrane filter of 0.22 μm maximum pore size. (**Warning**—Use the reagents in accordance with the appropriate health and safety regulations. Review their Material Safety Data Sheets before use.)

9. Procedure

9.1 Identify and document all areas to be sampled. Documentation should include:

9.1.1 General sampling site description.

9.1.2 Project or client name, address, and city/state location.

9.1.3 Sample location, which should include all information needed to locate the room and where the sample was collected. These include building, floor, room number, and room name.

NOTE 1—Some investigators include dimensions from some sort of reference (for example, 3 ft. 0 in. (0.9 m) from outside wall and 2 ft. 0 in. (0.6 m) from north wall), whereas others provide a section allowing such information to be recorded on a sample collection sheet.

9.1.4 Surface type, which should include descriptors of the surfaces in the room upon which the samples were collected. These include floor, wall, ceiling, top of light fixture, top of ceiling tile, exterior or duct, and so forth. It is sometimes useful to provide a section allowing for identification of surface sampled (for example, for a louver, whether the sample is from the top or bottom surface; for a grill, whether the sample is from the upstream or downstream side).

9.1.5 Surface material, which should describe the material from which the surface is constructed (for example, painted plaster or drywall, wood, concrete, metal, fabric, brick, resilient flooring, and so forth).

9.1.6 Surface description, which should describe the nature of the surface (for example color, texture, clean, dry, greasy, wet).

9.1.7 The area of surface wiped. It may not always be possible to collect from 100 cm^2 of surface. For example, one should indicate whether the effective surface area of a grill is

discounted for the open spaces in the grill.

9.1.8 Post sampling cleanliness of surface. A visual evaluation of the cleanliness of the surface post-sampling should be made and recorded. This evaluation should not be made until the surface has dried.

9.2 Two sampling procedures are presented (see EPA 747-R-95-001). One procedures for sampling in unrestricted areas such as floors (Template Assisted Sampling Procedure). The Confined Area Sampling Procedure should only be used when the Template Assisted Sampling Procedure can not be used due to sampling location constraints. The Confined Area Sampling Procedure assumes the width of the sampling location is no larger than the dimensions of a wipe. If this is not true, then the Template Assisted Sampling Procedure is used.

9.2.1 *Template Assisted Sampling Procedure*:

9.2.1.1 If a reuseable template is used, clean template (see 7.1.6).

9.2.1.2 Determine, measure, mark, or mask area, or place template onto surface. Document the location and area (cm^2) of surface to be sampled.

9.2.1.3 A typical sampling area is 100 cm^2. Smaller or larger areas may be sampled depending on surface cleanliness.

9.2.1.4 Put on a pair of clean, powderless, rubber gloves.

9.2.1.5 Adequately moisten the wipe with a 50/50 mixture of alcohol and water. For example, 10 to 20 mL will adequately moisten a 21 by 21 cm wipe. It is recommended that a portion of the wipe be tested with the mixture if there is any doubt that the solvent may damage the wipe material.

9.2.1.6 *First Wiping, Side to Side*—Hold one edge of the wipe between the thumb and forefinger, draping the wipe over the fingers of a gloved hand. Hold fingers together, hand flat, and wipe the selected surface area, starting at either corner furthest away from the operator (referred to as a far corner), and use a slow side to side (left to right or right to left) sweeping motion. During wiping, apply even pressure to the fingertips.

9.2.1.7 At the end of the first side to side pass, turn the wipe's leading edge (portion of the wipe touching the surface) 180°. Pull the wipe path slightly close to the operator and make a second side to side pass in the reverse direction, slightly overlapping the first pass. The 180° turn is used to ensure that the wiping motion is always performed in the same direction on the wipe to maximize sample pickup. Continue to cover the sampling area within the template, using the slightly overlapping side to side passes with the 180° turns at each edge until the close corner of the template is reached. Carefully lift the sampled material into the wipe, using a slight rolling motion of the hand to capture the sample inside the wipe. Fold the wipe in half with the sample folded inside the fold.

9.2.1.8 *Second Wiping, Top to Bottom*—Using a clean side of the wipe, perform a second wiping over the sampling area within the template, starting from a far corner in the same manner used for the first wiping, except use a top to bottom sweeping of the surface. When the close corner of the template is reached, carefully lift the sampled material into the wipe, using a slight rolling motion of the hand to capture the sample inside the wipe. Fold the wipe in half again, with the sample from this second wiping folded inside the fold.

9.2.1.9 *Third Wiping, Clean Corners*—Using a clean side of the wipe, perform a third wiping around the perimeter of the sampling area within the template. Start from one edge of the template and use the same wiping technique as described in 9.2.1.8. When the interior perimeter has been wiped and the starting location reached, carefully lift the sampled material into the wipe, using a slight rolling motion of the hand to capture the sample inside the wipe. Fold the wipe in half one more time, with the sample from this third wiping folded inside the fold.

9.2.1.10 Insert the folded wipe into a sample container and seal. Label the container with sample number and sufficient information to uniquely identify the sample.

9.2.1.11 If the template is a reusable type, clean the template (see 7.1.6).

9.2.1.12 Discard gloves.

9.2.1.13 Check that all sampling information sheets are completed and that all pertinent information has been enclosed before transferring the samples to the laboratory

9.2.1.14 Collect a field wipe (see 3.2.18).

9.2.1.15 Wipe off the exterior surface of the sample containers with disposable wet towels prior to packaging for shipment.

9.2.2 *Confined Area Sampling Procedure*:

9.2.2.1 Put on a pair of clean, powderless, rubber gloves.

9.2.2.2 Adequately moisten the wipe with a 50/50 mixture of alcohol and water. (For example, 10 to 20 mL will adequately moisten a 21 by 21 cm wipe.) It is recommended that a portion of the wipe be tested with the mixture if there is any doubt that the solvent may damage the wipe material.

9.2.2.3 *First Wiping, One Direction, Side-to-Side*—Hold one edge of the wipe between the thumb and forefinger, draping the wipe over the fingers of a gloved hand. Hold fingers together, hand flat, and wipe the selected surface area. Start at either corner furthest away from the operator (referred to as a far corner), and use a slow side to side (left to right or right to left) sweeping motion. During wiping, apply even pressure to the fingertips. At the end of the first pass from one side to the other, carefully lift the sample material into the wipe, using a slight rolling motion of the hand to capture the sample inside the wipe. Fold the wipe in half with the sample folded inside the fold.

9.2.2.4 *Second Wiping, One Direction, Side-to-Side*—Using a clean side of the wipe, repeat step 9.2.2.3, using a wiping motion in the reverse direction. When the close corner of the sampling area is reached, carefully lift the sampled material into the wipe, using a slight rolling motion of the hand to capture the sample inside the wipe. Fold the wipe in half again, with the sample from this second wiping folded inside the fold.

9.2.2.5 *Third Wiping, Clean Corners*—Using a clean side of the wipe, perform a third wiping around the interior perimeter of the sampling area. Start from the middle of one edge of an area and use the same wiping technique as described in 9.2.2.3. When the perimeter has been wiped and the starting location reached, carefully lift the sample material into the wipe, using a slight rolling motion of the hand to capture sample inside the wipe. Fold the wipe in half one more time with the sample from this third wiping folded inside the fold.

9.2.2.6 Insert the folded wipe into a sample container and seal. Label the sample container with sample number and sufficient information to uniquely identify the sample.

9.2.2.7 Discard gloves.

9.2.2.8 Using a tape measure, measure the dimension of the sampled surface with units such as inches or centimetres.

9.2.2.9 Check that all sampling information sheets are completed and that all pertinent information has been enclosed before transfer of the sample to the laboratory

9.2.2.10 Collect a field wipe.

9.2.2.11 Wipe off the exterior surface of the sample transport containers with disposable wet towels prior to packaging for shipment.

10. Sample Shipment

10.1 Ship samples to an analytical laboratory, separately packed from any bulk or air samples. The samples shall be packed in a material fiber-free material to minimize the potential for contamination.

NOTE 2—One package containing a large number of wipes moistened with a 50/50 mixture of alcohol and water may fall under regulations regarding transportation of dangerous goods. Prior to shipment, contact either International Air Transport Association (IATA) Dangerous Good Regulations for air shipment or Department of Transportation (DOT) for ground shipment.

11. Sample Suspension Preparation

11.1 Before taking sample containers into a clean preparation area, carefully wet-wipe the exterior of the containers to remove any possible contamination.

11.2 Perform sample preparation in a clean facility that has a separate work area from both the bulk and airborne asbestos sample preparation areas.

11.3 Initial specimen preparation (see 11.3.1-11.3.6) shall take place in a clean HEPA filtered negative pressure hood to avoid any possible contamination of the laboratory or personnel, or both, by the potentially large number of asbestos structures in an asbestos-containing surface wipe sample.

11.3.1 Transfer the wipe into a clean, wide-mouthed specimen bottle.

11.3.2 Rinse out the interior of the sample transport container with a known volume of water.

11.3.3 Pour this rinse water into the specimen bottle containing the wipe.

11.3.4 Add an additional measured volume into the labeled specimen bottle to submerge the wipe in 500 mL of water.

11.3.5 Using forceps, carefully unfold the wipe to expose all of the surfaces.

11.3.6 Adjust the pH of the suspension to 3 to 4, using a 10.0 % solution of acetic acid. Use pH paper for testing.

11.3.7 Replace the top to the specimen bottle, and lightly shake the suspension by hand for 3 s.

11.4 Place the specimen bottle in a tabletop ultrasonic bath. Maintain the water level in the sonicator at the same height as the suspension in the specimen bottle. Sonicate for 5.0 min to release particles from wipe.

11.4.1 Calibrate the ultrasonic bath as described in 21.4.2. Operate the ultrasonic bath at equilibrium temperature. After sonication, return the specimen bottle to the work surface of the HEPA hood.

12. Blank Filtration

12.1 Process at least one field wipe (see 3.2.18) along with each batch of samples to test for potential contamination during the sampling, shipping, handling, and preparation steps of the test method. Reject the sample set or take appropriate actions if relatively high fiber counts are determined.

12.2 In addition, process sample blanks that include a process blank (see 3.2.25) and filtration blank (see 3.2.19). If glass filtration units are used, prepare a filtration blank prior to each new use of the filtration unit.

12.3 The process and filtration blanks will be considered contaminated if, after analysis, they are shown to contain more than 53 asbestos structures per square millimetre of the analyzed filter. This generally corresponds to 3 or 4 asbestos structures found in 10 grid squares. The source of the contamination must be found before any further analysis can be performed. Reject samples that were processed along with the contaminated blanks, and prepare new samples after the source of the contamination is found.

13. Sample Filtration

13.1 Use a filtration unit for filtration of suspension aliquots.

13.2 If a disposable plastic filtration unit is used, then unwrap a new disposable plastic filter funnel unit and remove the tape around the base of the funnel. Remove the funnel and discard the top filter supplied with the apparatus. Retain the coarse polypropylene support pad in place. Assemble the unit with the adapter and a properly sized neoprene stopper, and attach the funnel to the 1000-mL side arm vacuum flask. Place a 5.0-µm pore size MCE (backing filter) on the support pad.

13.3 Wet the backing filter with a few millilitres of water, and place a ≤0.22-µm MCE or a ≤0.2-µm PC filter on top of the backing filter. Apply a vacuum, ensuring that the filters are centered and pulled flat without air bubbles. Any irregularities on the filter surface require the discard of the ≤0.22-µm MCE or the ≤0.2-µm PC filter.

13.4 Once the filter has been seated properly, replace the funnel and reseal it with the tape. Return the flask to atmospheric pressure.

NOTE 3—When using a PC filter, the filter must not be allowed to dry before filtration. PC filters are hydrophobic. A water-soluble wetting agent is applied to the surface in order to make the surface hydrophilic. Once this agent is removed and the filter allowed to dry, filtration through the PC filter is almost impossible.

13.5 If a glass filtration unit is used, place a 5-µm pore size MCE (backing filter) on the glass frit surface.

13.6 Wet the backing filter with a few mL of water, and place an MCE or PC filter (≤ 0.22-µm pore size) on top of the backing filter. Apply a vacuum, ensuring that the filters are centered and pulled flat without air bubbles. Replace the filters if any irregularities are seen on the filter surface.

13.7 If aliquots of the same sample are filtered in order of increasing concentration or volume, the glass filtration unit need not be washed between filtration.

13.8 After completion of the filtration, do not allow the

filtration funnel assembly to dry because contamination is then more difficult to remove. Wash any residual suspension from the filtration assembly by holding it under a flow of water, and then rub the surface with a clean paper towel soaked in a detergent solution. Repeat the cleaning operation, and then rinse two times in water.

13.9 With the flask at atmospheric pressure, add 20 mL of water into the funnel. Cover the filter funnel with its plastic cover if the disposable filtering unit is used.

13.10 Shake the sample suspension lightly by hand for 3 s, then let it rest for 2.0 min to allow large particles to settle to the bottom of the bottle or float to the surface.

13.11 Insert a new pipette into the sample suspension to withdraw an aliquot from the central region of the suspension. Avoid pipetting any of the large floating or settledparticles. Uncover the filter funnel and dispense the aliquot from the pipette into the water in the funnel. Stir with pipette to mix thoroughly.

13.11.1 Estimate the amount of suspension to be withdrawn to produce an adequate filter preparation. A light staining of the filter surface will yield a suitable preparation for analysis. Filter at least 1.0 mL, but no more than half the total volume.

13.11.2 To ensure that an optimally loaded filter is obtained, it is recommended that filters be prepared from varying aliquots of the suspension. A 3 to 10 % particulate coverage of the grid opening is ideal.

13.11.2.1 If the filters are prepared in order of increasing aliquot volume, all of the filters for one sample can be prepared using one plastic disposable filtration unit, or without cleaning of glass filtration equipment between individual filtration. Before withdrawal of each aliquot from the sample, shake the suspension without additional sonification and allow to rest for 2 min.

13.11.3 If after examination in the TEM, the smallest volume measured (1.0 mL) yields an overloaded sample, perform a sample dilution.

13.11.3.1 If a sample dilution is required, repeat 13.10 before the dilution aliquot is taken. Do not resonicate the original solution or any sample dilutions. Mix 10 mL of the sample suspension with 90 mL of water in a clean specimen bottle to obtain a 1:10 dilution. Follow good laboratory practices when performing dilutions.

13.12 Apply vacuum to the flask, and draw the suspension through the filter.

13.13 Discard the pipette.

13.14 Disassemble the filtering unit, and carefully remove the sample filter with fine forceps. Place the completed sample filter, sample surface side up into a precleaned, labeled disposable plastic petri dish or other similar container.

13.15 There are many practical methods for drying both MCE and PC filters, for example, drying filters in a plastic petri dish on a slide warmer or in a low temperature oven at 65 to 70°C for 10 to 15 min.

13.16 Prepare TEM specimens from small sections of each dried filter, using the appropriate direct transfer preparation method (see Sections 14 and 15).

14. TEM Specimen Preparation of Mixed Cellulose Ester (MCE) Filters

NOTE 4—Use of either the acetone or the dimethyl formamide (DMF)-acetic acid method is acceptable.

14.1 *Acetone Fusing Method*:

14.1.1 Process at least one filter blank with every batch of samples.

14.1.2 Remove a section from any quadrant of the sample and blank filters. Sections can be removed from the filters using either a scalpel or 7-mm cork borer. The scalpel or cork borer must be wet- wiped after each time a section is removed.

14.1.3 Place the filter section (sample 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.

14.1.4 Prepare a fusing dish from a glass petri dish and a metal screen bridge with a pad of five to six paper filters, and place in the bottom of the petri dish (see 40 CFR 763). Place the screen bridge on top of the pad and saturate the filter pads with acetone. 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.

14.2 *DMF-Acetic Acid Method*:

14.2.1 Process at least one filter blank with every batch of samples.

14.2.2 Place a drop of clearing solution that consists of 35 % DMF, 15 % glacial acetic acid, and 50 % water (v/v) on a clean microscope slide. Gage the amount used so that the clearing solution just saturates the filter section.

14.2.3 Carefully lay the filter segment, sample surface upward, on top of the solution. Bring the filter and solution together at an angle of about 20° to help exclude air bubbles. Remove any excess collapsing solution by allowing an absorbent tissue to contact the liquid at the edge of the filter. Place the slide in an oven or on a hot plate, in a fume hood, at 65 to 70°C for 10 min.

14.3 *Plasma Etching of the Collapsed Filter*:

14.3.1 The plasma asher shall be calibrated as described in 21.4.4.

14.3.2 The microscope slide to which the collapsed filter pieces are attached is placed in a plasma asher.

14.3.3 Position the slide with portions of collapsed MCE filter approximately in the center of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8 to 20 cm^3/min. Adjust the tuning of the system so that the intensity of the plasma is maximized.

14.3.4 Place the glass slide containing the collapsed filters into the plasma asher, and etch the filter using the optimum conditions and time. Admit air slowly to the chamber after etching, and remove the microscope slide.

14.3.4.1 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.

14.4 *Carbon Coating of the Collapsed and Etched Filters*:

14.4.1 Carbon coating shall be performed with a high-vacuum coating unit, capable of less than 10^{-4} torr (13 mPa)

pressure. Units that are based on evaporation of carbon filaments in a vacuum generated only by an oil rotary pump have not been evaluated for this application and shall not be used. Carbon rods used for evaporators shall be sharpened with a carbon rod sharpener to a neck of about 4 mm in length and 1 mm in diameter. The rods are installed in the evaporator in such a manner that the points are approximately 100 to 120 mm from the surface of the microscope slide held in the rotating device.

14.4.2 Place the glass slide holding the filters on the rotation device, and evacuate the evaporator chamber to a vacuum of at least 13 mPa. Perform the evaporation in very short bursts, separated by 3 to 4 s to allow the electrodes to cool An alternate method of evaporation is by using a slow continuous applied current. An experienced analyst can judge the thickness of the carbon film to be applied. Conduct tests on unused filters first. If the carbon film is too thin, large particles will be lost from the TEM specimen, and there will be few complete and undamaged grid openings on the specimen. If the coating is too thick, it will lead to a TEM image that is lacking in contrast, and the ability to obtain electron diffraction patterns will be compromised. The carbon film shall be as thin as possible and still remain intact on most of the grid openings of the TEM specimen.

14.5 *Preparation of the Solvent Washer (Jaffe Washer)*—The precise design of the Jaffe washer is not considered important, so any one of the published designs may be used (5) (see EPA 600/4-83-043). One such washer consists of a simple stainless steel bridge contained in a glass petri dish.

14.5.1 Place several pieces of lens tissue on the stainless steel bridge. The pieces of lens tissue shall be large enough to completely drape over the bridge and into the solvent. In a fume hood, fill the petri dish with acetone or DMF until the height of the solvent is brought up to contact the underside of the metal bridge as illustrated in Fig. 1 (see EPA 600/4-83-043).

14.6 Using a curved scalpel blade, excise at least 3 by 3-mm square pieces of the carbon-coated MCE filter from the glass slide. Place the square filter piece carbon-side up onto the shiny side of a TEM specimen grid. Place the whole filter/grid assembly onto the saturated lens tissue in the Jaffe washer.

14.7 Prepare three specimen grids from each sample.

14.8 Alternately, place the grids on a low level (petri dish filled to the ⅛ mark) DMF Jaffe washer for 60 min. Add enough solution of equal parts DMF/acetone to fill the washer to the screen level. Remove the grids after 30 min if they have *cleared*, that is, all filter material has been removed from the carbon film, as determined by inspection in the TEM.

14.9 Carefully remove the grids from the Jaffe washer, allowing the grids to dry before placing them in a clean, labeled grid box.

14.10 Place the lid on the Jaffe washer, and allow the system to stand for several hours.

15. TEM Specimen Preparation of Polycarbonate (PC) Filter

15.1 Use a cleaned microscope slide to support representative portions of the PC filter during carbon evaporation. Apply two parallel strips of double-sided adhesive tape along the length of the slide, separated by a distance of approximately 22 mm.

15.2 Using a clean, curved scalpel blade, cut a strip of the PC filter approximately 25 by 6 mm. Use a rocking motion of the scalpel blade to avoid tearing the filter. Place the PC strip particle side up on the slide perpendicular to the long axis of the slide. The ends of the PC strip shall contact the double-sided adhesive tape. Each slide can hold several PC strips. With a glass marker, label next to each PC strip with the individual sample number.

15.3 PC filters do not require etching. Carbon coat the PC filter strips as discussed in 14.4. (**Warning**—Do not overheat the filter section while carbon coating.)

15.4 Prepare a Jaffe washer as described in 14.5, but fill the washer with chloroform to the level of the screen.

15.4.1 Using a clean curved scalpel blade, excise three 3 by 3 mm square filter pieces from each carbon-coated PC strip. Place the filter squares carbon side up onto the shiny side of a TEM grid. Place the whole filter/grid assembly onto the lens tissue in the Jaffe washer.

15.4.2 Prepare three specimen grids from each sample.

15.4.3 Place the lid on the Jaffe washer, and allow to stand for at least 4 h. Best results are obtained with longer times, up to 12 h.

15.5 Carefully remove the grids from the Jaffe washer, allowing the grids to dry before placing them in a clean labeled grid box.

15.6 *1-methyl-2-pyrrolidone Method*:

15.6.1 Prepare a Jaffe washer as described in 14.5, but fill the washer with a mixture of 20 %1,2–diaminoethane (ethylene diamine) and 80 % 1-methyl-2-pyrrolidone to the level of the screen.

15.6.2 Using a cleancurved , scalpel blade, excise three 3 by 3 mm square filter pieces from each PC strip. Place the filter squares carbon side up on the shiny side of a TEM grid. Pick up the grid and filter section together, and place them directly on the stainless steel mesh of the Jaffe washer (do not use lens paper) for 15 min.

15.6.3 Prepare three specimen grids from each sample.

15.6.4 Transfer the stainless steel bridge into another petri dish, and add distilled water until the meniscus contacts the underside of the mesh. Wait 15 min.

15.7 Remove mesh, and allow grids to dry before placing them in a clean, marked grid box.

16. Grid Opening Measurements

16.1 TEM grids shall have a known grid opening area. Determine this area by one of the following methods:

16.1.1 Measure at least 20 grid openings in each of 20 random 200-mesh electron microscope grids for a total of 400 grid openings for every 1000 grids used, by placing the 20 grids on a glass slide and examining them under the optical microscope. Use a calibrated graticule to measure the average length and width of the 20 openings from each of the individual grids. From the accumulated data, calculate the average grid opening area of the 400 openings.

16.1.2 Grid opening area measurements can also be made at the TEM at a suitable calibrated screen magnification such that an entire grid opening is visible within the calibration area of

the screen at one time. Typically, one grid opening for each grid examined is measured. Measure grid openings in both the *X* and *Y* directions, and calculate the area.

16.1.3 Commercially available precalibrated TEM grids are also acceptable for this test method.

17. TEM Examination

17.1 *Microscope Settings*—80 to 120 kV, 15 000 to 20 000 X screen magnification for analysis.

17.2 For the analyses, analyze two grids for each sample. Select approximately half of the grid openings to be examined from each of the two grids.

17.3 *Determination of Specimen Suitability*—Use a hand lens or loupe if necessary. Carefully load the TEM grid into the specimen holder so that the carbon side will be facing down in the TEM column, with the grid bars oriented parallel/perpendicular to the length of the specimen holder. This procedure will line up the TEM grid with the *X* and *Y* translation directions of the microscope. Insert the specimen holder into the microscope.

17.3.1 Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Examine the specimen grid in the TEM at a sufficiently low magnification (300 to 1 000 X) so that complete grid opening can be inspected. Reject the grid if:

17.3.1.1 The specimen grid 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, perform additional solvent washing, or prepare new specimens from the filter;

17.3.1.2 The sample is over-loaded with particulate matter. If the specimen grid exhibits more than approximately 10 % obscuration on the majority of the grid openings, designate the specimen preparation as over-loaded. This filter cannot be satisfactorily analyzed 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 under-estimation of the asbestos structure count;

17.3.1.3 The particulate loading on the specimen is not uniformly distributed from one grid opening to the next. If the particulate loading on the specimen is obviously not uniform from one grid to the next, designate the specimen as nonuniform. Satisfactory analysis of this filter may not be possible unless a large number of grid openings are examined;

17.3.1.4 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 30 asbestos fibers per grid opening; or

17.3.1.5 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 the intact grid openings can lead to an underestimate of the structure count.

17.3.2 If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carton replica, apply an additional carbon coating to the carbon-coated filter, and prepare new specimen grids. A thicker carbon film can often support the larger particles. However, too thick a carbon film will reduce image contrast and have an adverse effect on the analyst's ability to discern weak diffraction patterns from thin fibers.

17.4 *Data Recording Rules*:

17.4.1 Observe and record the orientation of the grid at 80 to 150 X on a grid map record sheet along with the location of the grid openings that are examined for the analysis. If indexed grids are used, a grid map is not required, but the identifying coordinates of the grid openings must be recorded.

17.4.2 Record on the count sheet any continuous grouping of particles in which an asbestos fiber is detected. Classify asbestos structures as fibers, bundles, clusters, or matrices as defined in 3.2.

17.4.3 Use the criteria for fiber, bundle, cluster, and matrix identification, as described in 40 CFR 763. Record the length and width measurements for each identified Asbestos Hazard Emergency Response Act (AHERA) structure.

17.4.4 Record NSD (No Structures Detected) when no AHERA structures are detected in the grid opening.

17.4.5 Identify structures classified as chrysotile by either ED or EDXA, and record on a count sheet. Verify at least one out of every ten chrysotile structures by either ED or EDXA.

17.4.6 Structures classified as amphiboles by EDXA and ED are recorded on the count sheet. For more information on identification, see Ref. (5) or EPA 600/4-83-043.

17.4.7 Record a typical electron diffraction pattern for each type of asbestos observed for each group of samples (or a minimum of every five samples) analyzed. Record the micrograph number on the count sheet. Record at least one X-ray spectrum for each type of asbestos observed per sample. If the X-ray spectrum is stored, record the file and disk number on the count sheet. If a hard copy is generated, retain the hard copy with the count sheets.

17.5 *Counting Rules*:

17.5.1 At a screen magnification of between 15 000 and 20 000 X, evaluate the grids for the most concentrated sample loading. Reject the sample if it is estimated to contain more than 50 asbestos structures per grid opening. Proceed to the next lower concentrated sample unit a set of grids are obtained that have less than 30 asbestos structures per grid opening.

17.5.2 *Analytical Sensitivity*—An analytical sensitivity of approximately 260 asbestos structures per square centimetre (calculated for the detection of a single asbestos structure) has been designed for this analysis. This sensitivity can be achieved by increasing the amount of liquid filtered, increasing the number of grid openings analyzed or decreasing the size of the final filter. Occasionally, due to high particle loading or high asbestos concentration, this analytical sensitivity cannot be practically achieved and stopping rules apply (see 17.6).

17.6 *Stopping Rules*:

17.6.1 A minimum of four grid openings shall be analyzed for each sample.

17.6.2 The nominal number of grid openings to be analyzed is the number required to achieve an analytical sensitivity of 260 asbestos structures per square centimetre. If, due to dilution or any other reason, the analytical sensitivity is not achievable by analyzing ten grid openings, stop at the grid opening that contains the hundreth asbestos structure, or

completion of the tenth grid opening, whichever comes first.

17.7 After analysis, remove the grids from the TEM and replace them in the appropriate labeled grid box.

18. Sample Storage

18.1 The washed-out sample containers can be discarded after use.

18.2 Sample grids and unused filter section shall be stored for a minimum of one year.

19. Report

19.1 Report the following information for each sample analyzed:

19.1.1 Concentration of asbestos structures per square centimetre of area wiped,

19.1.2 Analytical sensitivity,

19.1.3 Detection limit,

19.1.4 Types of asbestos present,

19.1.5 Number of asbestos structures counted,

19.1.6 Effective filtration area of filter through which the suspensions were drawn (mm^2),

19.1.7 Average area of the TEM grip opening (mm^2),

19.1.8 Number of grid openings examined,

19.1.9 Initial volume of water introduced into specimen bottle containing wipe, see 11.3,

19.1.10 Volume of suspension filtered,

19.1.11 Area of surface wiped,

19.1.12 Listing of size data for each structure counted, and

19.1.13 A copy of the TEM count sheet or a complete listing of the raw data. An example of a typical count sheet is shown in Appendix X1.

19.2 Determine the amount of asbestos in any accepted sample, using the following formula:

$$\frac{EFA\ IV\ \#STR}{GO\ GOA\ V\ SA} = \text{Asbestos Structures/cm}^2 \qquad (2)$$

where:

#STR = number of asbestos structures counted,
EFA = effective filtration area of filter through which the suspension were drawn, mm^2,
GO = number of grid openings counted,
GOA = average grid opening area, mm^2,
SA = area of surface wiped (cm^2),
V = volume of suspension filtered in 10.8, mL, and
IV = initial volume, mL. Measured volume of water introduced into the specimen bottle containing the wipe (see 10.3.4).

20. Quality Control/Quality Assurance

20.1 In general, the laboratory's quality control checks are used to verify that a system is performing in accordance with specifications regarding accuracy and consistency. In an analytical laboratory, spiked or known quantitative samples are normally used. At this time, due to the difficulties in prepared known quantitative asbestos samples, the accuracy of this test method cannot be determined. However, routine quality control testing focusing on reanalysis of samples is to be performed.

20.1.1 Reanalyze samples at a rate of 1/10 of the sample sets (one out of every ten samples analyzed, not including blanks). The reanalysis shall consist of a second specimen grid preparation.

20.2 In addition, quality assurance programs shall follow the criteria shown in 40 CFR 763 and in Ref. (6). These documents describe sample custody, sample preparation, and blank checks for contamination, calibration, sample analysis, analyst qualifications, and technical facilities.

21. Calibrations

21.1 Perform calibrations of the instrumentation on a regular basis, and retain these records in the laboratory in accordance with the laboratory's quality assurance program.

21.2 Record calibrations in a logbook, along with calibration dates and data.

21.3 Frequency of calibration will depend on the service history of the particular instrument/equipment.

21.4 *Calibration List for Instruments Follows*:

21.4.1 *TEM*:

21.4.1.1 Check the alignment and systems operation. Refer to the TEM manufacturer's operational manual for detailed instructions.

21.4.1.2 Check the calibration after any maintenance of the microscope that involves adjustment of the power supply to the lens or the high voltage system or the mechanical disassembly of the electron optical column (apart from filament exchange).

21.4.1.3 Calibrate the camera length of the TEM in ED operating mode before ED patterns of unknown samples are observed. Camera length can be measured by using a carbon-coated grid on which a thin film of gold has been sputtered or evaporated. A thin film of gold is evaporated on the specimen TEM grid to obtain zone-axis ED patterns superimposed with a ring pattern from the polycrystalline gold film. In practice, it is desirable to optimize the thickness of the gold film so that only on or two sharp rings are obtained on the superimposed ED pattern. Thick gold films will tend to mask weak diffraction spots from the fibrous particles. Since the unknown *d*-spacings of most interest in asbestos analysis are those that lie closest to the transmitted beam, multiple gold rings from thick films are unnecessary. Alternatively, a gold standard specimen can be used to obtain an average camera constant calculated for that particular instrument and can then be used for ED patterns of unknowns taken during the corresponding period.

21.4.1.4 Perform magnification calibration at the fluorescent screen. This calibration shall be performed at the magnification used for structure counting. Calibration is performed with a grating replica.

(*a*) Define a field of view on the fluorescent screen. The field of view shall be measurable or previously inscribed with a scale or concentric circles (all scales should be metric).

(*b*) The analyst shall ensure that the grating replica is placed at the same distance from the objective lens as the specimen.

(*c*) For instruments that incorporate an eucentric tilting specimen stage, all specimens and the grating replica shall be placed at the eucentric position.

21.4.1.5 The dimension of the smallest spot size routinely used for EDXA analysis shall be measured.

(*a*) At the crossover point, photograph the spot size at a screen magnification of 15 000 to 20 00 X. An exposure time of 1 s is usually adequate.

(*b*) The measured spot size shall be less than or equal to 250 nm.

21.4.2 *EDXA*:

21.4.2.1 The EDXA detector resolution (at the MnKα peak) and energy calibration shall be determined and monitored over time.

(*a*) Calibrate the EDXA using at least two peaks between 0.7 and 10 keV. One peak should be from the low end (0.7 to 2 keV) and the other peak form the high end (7 to 10 keV).

(*b*) Compare the X-ray energy versus the channel number, and adjust equipment if reading is not within ± 10 eV.

21.4.2.2 Collect a standard EDXA of crocidolite (NIST SRM 1866). The elemental analysis of the crocidolite shall resolve the Na peak.

21.4.2.3 Collect a standard EDXA of chrysotile (NIST SRM 1866). The elemental analysis of chrysotile shall resolve both Si and Mg from a single chrysotile fiber.

21.4.3 *Ultrasonic Bath Calibration*:

21.4.3.1 Fill the bath water to a level equal to the height of suspension in the glass sample container that will be used for sample suspension dispersion. Operate the bath until the water reaches the equilibrium temperature.

21.4.3.2 Place 100 mL of water (at approximately 20°C) in a 200 mL glass sample container, and record the water temperature.

21.4.3.3 Place the sample container in the water in the ultrasonic bath (with the power turned off). After 60 s, remove the glass container and record the water temperature.

21.4.3.4 Place 100 mL of water (approximately 20°C) in another 200 mL glass sample container, and record the water temperature.

21.4.3.5 Place the second sample container into the water in the ultrasonic bath (with the power turned on). After 60 s, remove the glass container and record the water temperature.

21.4.3.6 Calculate the rate of energy deposition into the sample container using the following formula:

$$R = 4.185\, S\, D\, \frac{(q_1 - q_2)}{t} \quad (3)$$

where:

4.185 = joules/calorie,
R = energy deposition, W/mL,
q_1 = temperature rise with the ultrasonic bath not operating °C
q_2 = temperature rise with the ultrasonic bath operating, °C
t = time, s (see 21.4.3.3),
S = specific heat of the liquid in the glass sample container (0.2389 J/g), and
D = density of the liquid in the glass sample container (1.0 g/cm^3).

21.4.3.7 Adjust the operating conditions of the bath so that the rate of energy deposition is in the range from 0.08 to 0.12 MW/m^3, as defined by this procedure.

21.4.4 *Plasma Asher Calibration*:

21.4.4.1 Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the exact conditions that shall be used. Insufficient etching will result in a failure to expose embedded fibers, and too much etching may result in the loss of particles from the filter surface. To determine the optimum time for ashing, determine a calibration curve for the weight versus etching time of collapsed MCE filters.

(*a*) Place a microscope slide containing a collapsed, 25-mm diameter MCE filter into the center of the asher chamber.

(*b*) Closes the chamber and evacuate to a pressure of 40 Pa, while admitting oxygen to the chamber at a rate from 8 to 20 mL/min. Adjust the tuning of the system so that the intensity of the plasma is maximized.

(*c*) Measure the time required for complete oxidation of the filter.

(*d*) 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. Weigh the filter after etching.

(*e*) Adjust the parameters and retest until a 1 to 10 % weight loss is achieved.

(*f*) The AHERA (see 40 CFR 763) method specifies that a MCE filter is to be etched by 10 %. However, if this amount generates a texture in the replica that affects structure counting, it is permissible to etch by less than this amount **(6)**. The final acceptance of the etched filter is dependent on its appearance in the TEM.

22. Precision and Bias

22.1 *Precision*—To be determined.

22.2 *Bias*—To be determined (see Guide D 3670).

23. Keywords

23.1 asbestos; surface sampling; TEM; wipe sampling

APPENDIX

(Nonmandatory Information)

X1. SAMPLE ANALYSIS

X1.1 See Fig. X1.1 for the analysis work sheet.

X1.2 See Fig. X1.2 for the TEM count sheet.

SAMPLE ANALYSIS

Client: _____
Sample ID: _____
Job Number: _____
Date Sample Analyzed: _____ - _____ - _____
Number of Openings/Grids Counted: _____ / _____
Grid Accepted, 600X: Yes No
Percent Loading: _____ %
Grid Box #1: _____

Analyst: _____

Reviewer: _____

Accelerating Voltage: _____
Indicated Mag: _____ KX
Screen Mag: _____ KX
Microscope: 1 2 3 4 5
Filter Type: _____
Filter Size: _____
Filter Pore Size (µm): _____
Grid Opening: 1) _____ µm x _____ µm
2) _____ µm x _____ µm

Calculation Data:

Effective Filter Area (mm^2):	(EFA)	_____
Number of Grid Openings Counted:	(GO)	_____
Average Grid Opening Area (mm^2):	(GOA)	_____
Volume of sample Filtered (ml):	(V)	_____
Surface Area Sampled (cm^2):	(SA)	_____
Initial Volume (ml)	(IV)	_____
Number of Asbestos Structures Counted:	(#STR)	_____

FORMULA FOR CALCULATION OF ASBESTOS STRUCTURES PER CM^2 OF SURFACE WIPED:

$$\frac{(EFA)(IV)(\#STR)}{(GO)(GOA)(V)(SA)} = \text{Asbestos Structures per } cm^2$$

Results for Total Asbestos Structures: (Structures per cm^2)

FIG. X1.1 Analysis Work Sheet

Job Number: ____________________

Structure #	Grid Opening#	Type	Structure	Length (µm)	Width (µm)	Confirmation		
						Morph	ED	EDXA

Note: Keys to Abbreviations Used in Figure:

Type:

C = Chrysotile
AM = Amosite
CR = Crocidolite
AC = Actinolite
TR = Tremolite
AN = Anthophyllite
N = Non Asbestos

Structure:

F = Fiber
B = Bundle
C = Cluster
M = Matrix

Others:

NSD = No Structures Detected
Morph = Morphology
ED = Electron Diffraction
EDXA = Energy Dispersive X-Ray Analysis

FIG. X1.2 TEM Count Sheet

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