



Designation: **D6480 – 05 (Reapproved 2010) D6480 – 19**

Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Surface Loading by Transmission Electron Microscopy¹

This standard is issued under the fixed designation D6480; 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 surface loading 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 The values stated in SI units are to be regarded as standard.

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

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

2. Referenced Documents

2.1 ASTM Standards:²

[D1193 Specification for Reagent Water](#)

[D1356 Terminology Relating to Sampling and Analysis of Atmospheres](#)

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 Sampling, Analysis, Management of Asbestos, and Other Microscopic Particles.

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² 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.

[E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method](#)
[D3670E177 Guide Practice for Determination Use of the Terms Precision and Bias of Methods of Committee D22 in ASTM Test Methods](#)

2.2 *Government Standard:*³

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

2.3 *U.S. Environmental Protection Agency Standards:*³

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 [D1356](#).

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *amphibole asbestos*—*asbestos, n*—amphibole in an asbestiform habit **(1)**.⁴

3.2.2 *analytical sensitivity*—*sensitivity, n*—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*—*asbestos, n*—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 **(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.⁴

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

⁴ The nonasbestiform variations of the minerals indicated in 3.2.3.1 have different Chemical Abstract Service (CAS) numbers.

3.2.4 *asbestos structure*—*structure, n*—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*—*ratio, n*—the length to width ratio of a particle.

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

3.2.8 *chrysotile*—*chrysotile, n*—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*—*cluster, n*—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*—*spacing, n*—the perpendicular distance between identical adjacent and parallel planes of atoms in a crystal.

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

³ Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401-20401-0001, <http://www.access.gpo.gov>.

⁴ The boldface numbers in parentheses refer to the list of references at the end of this standard.

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

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

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

3.2.18 *field wipe blank*—*blank, n*—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*—*blank, n*—an unused, unprocessed filter of the type used for liquid filtration.

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

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

3.2.22 *indirect preparation*—*preparation, n*—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*—*detection, n*—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*—*matrix, n*—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*—*blank, n*—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*—*sampling, n*—one of several identical procedures or samples.

3.2.27 *serpentine*—*serpentine, n*—a group of common rock-forming minerals having the nominal formula: $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$. For further information see Ref. Ref (4).

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

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

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

3.3 Symbols:

<i>eV</i>	= electron volt
<i>h</i>	= hour
<i>J</i>	= joule
<i>kV</i>	= kilovolt
<i>min</i>	= minute(s)
<i>mL</i>	= 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 to ~~20 000~~ 20 000×

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 surface loading 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 (attapulgitite),
- 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 1/8 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 μ L.

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~~ 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 ≤ 0.22 and 5- μ m 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 - μ m diameter, ≤ 0.2 - μ m 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.

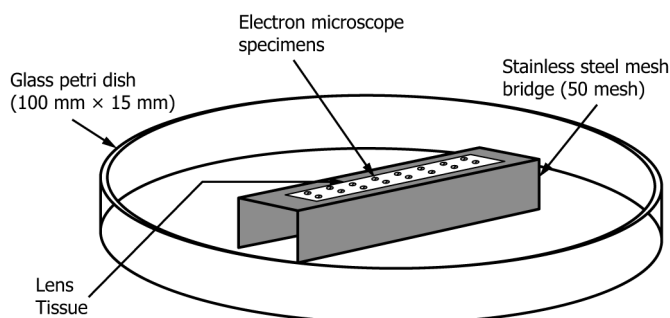


FIG. 1 Example of Solvent Washer (Jaffee Wick Washer)

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 MnK α 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 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 NaK α 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 \times shall be used, with the ability to obtain a screen magnification of about 100 000 \times , 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~~ 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:

- (1) Rotated through 360 $^\circ$, combined with tilting through at least +30 to -30 $^\circ$ about an axis in the plane of the specimen; or
- (2) Tilted through at least +30 to -30 $^\circ$ 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