Designation: D 5756 – 95

Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Mass Concentration[1]

This standard is issued under the fixed designation D 5756; 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 (*a*) identify asbestos in dust and (*b*) provide an extimate of the concentration of asbestos in the sampled dust, reported as either the mass of asbestos per unit area of sampled suurface or as the mass of asbestos per mass of sampled dust.

1.1.1 If an estimate of asbestos structure counts is to be determined, the user is referred to Test Method D 5755.

1.2 This test method describes the equipment and procedures necessary for sampling, by a microvacuum technique, non-airborne dust for levels of asbestos. The non-airborne sample is collected inside a standard filter membrane cassette from the sampling of a surface area for dust which may contain asbestos.

1.2.1 This procedure uses a microvacuuming sampling technique. The collection efficiency of this technique is unknown. Variability of collection efficiency for any particular substrate and across different types of substrates is also unknown. The effects of sampling efficiency differences and variability on the interpretation of dust sampling measurements have not been determined.

1.3 Asbestos identified by transmission electron microscopy (TEM) is based on morphology, selected area electron diffraction (SAED), and energy dispersive X-ray analysis (EDXA). Some information about structure size is also determined.

1.4 This test method is generally applicable for an estimate of the concentration of asbestos starting from approximately 0.24 pg of asbestos per square centimeter (assuming a minimum fiber dimension of 0.5 µm by 0.025 µm, see 17.8), but will vary with the analytical parameters noted in 17.8.

1.4.1 The procedure outlined in this test method employs an indirect sample preparation technique. It is intended to disaggregate and disperse asbestos into fibrils and fiber bundles that can be more accurately identified, counted, and sized by transmission electron microscopy. However, as with all indirect sample preparation techniques, the asbestos observed for quantitation may not represent the physical form of the asbestos as sampled. More specifically, the procedure described nmeither creates not destroys asbestos, but it may alter the physical form of the mineral fibers.

1.5 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

D 1739 Test Methods for Collection and Measurement of Dustfall (Settleable Particulate Matter)[3]

D 3195 Practice for Rotameter Calibration[3]

D 5755 Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Concentrations[3]

E 832 Specification for Laboratory Filter Papers[4]

2.2 *ISO Standards:*

ISO/10312 Ambient Air: Determination of Asbestos Fibers; Direct Transfer Transmission Electron Microscopy Procedure[5]

ISO/CD13794 Ambient Air: Determination of Asbestos Fibres; Indirect-Transfer Transmission Electron Microscopy Procedure[5]

3. Terminology

3.1 *Definitions:*

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

[1] This test method is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis of Atmospheresand is the direct responsibility of Subcommittee D22.07on 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] *Annual Book of ASTM Standards*, Vol 14.02.

[5] Available from American National Standards Institute, 11 W. 42nd St., 13th Floor, New York, NY 10036.

information on aasbestiform mineralogy, see references **(1)**, **(2)** and **(3)**.[6]

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

3.1.2.1 *Discussion*—Included in the definition are the asbestiform varieties of: serpentine (chyrsotile); riebeckite (crocidolite); grunerite (amosite); anthophyllite (anthophyllite asbestos); tremolite (tremolite asbestos); and actinolite (actinolite asbestos). The amphibole mineral compositions are defined according to the nomemclature of the International Mineralogical Association **(3)**.

Asbestos	Chemical Abstract Service No.[7]
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

3.1.3 *fibril*—a single fiber that cannot be separated into smaller components without losing its fibrous properties or appearance.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aspect ratio*—the ratio of the length of a fibrous particle to its average width.

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

3.2.3 *cluster*—an aggregate of two or more randomly oriented fibers, with or without bundles. Clusters occur as two varieties—disperse clusters and compact clusters.

3.2.3.1 *compact cluster*—a complex and tightly bound network in which one or both ends of each individual fiber or bundle are obscured, such that the dimensions of individual fibers or bundles cannot be unambiguously measured.

3.2.3.2 *disperse cluster*—a disperse and open network in which both ends of one of the individual fibers or bundles can be separately located and its dimensions measured.

3.2.4 *debris*—materials that are of an amount and size (particles greater than 1 mm in diameter as defined by a 1.0 by 1.0 mm screen) that can be visually identified (by color, texture, etc.) as to their source.

3.2.5 *dust*—any material composed of particles in a size range of ≤1 mm and large enough to settle by virtue of their weight from the ambient air. See Test Method D 1739.

3.2.6 *fiber*—a structure having a minimum length of 0.5µ m with an aspect ratio of 5 to 1 or greater and substantially parallel sides **(4)**. Fibers are assumed to have a cylindrical shape **(5)**.

3.2.7 *fibrous mineral*—a mineral that is composed of parallel, radiating, or interlaced aggregates of fibers, from which the fibers are sometimes separable.

3.2.7.1 *Discussion*—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.

3.2.8 *indirect preparation*—a method in which a sample passes through one or more intermediate steps prior to final filtration.

3.2.9 *matrix*—a structure in which one or more fibers, or fiber bundles, touch, are attached to, or partially concealed by a single particle or connected group of non-fibrous particles. The exposed fiber must meet the fiber definition (see section 3.2.6). Matrices occur as two varieties—disperse matrices and compact matrices.

3.2.9.1 *compact matrix*—a structure consisting of a particle or linked group of particles, in which fibers or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibers and bundles cannot be unambiguously determined.

3.2.9.2 *disperse matrix*—a structure consisting of a particle or linked group of particles, with overlapping or attached fibers or bundles in which at least one of the individual fibers or bundles can be separately identified and its dimensions measured.

3.2.10 *structures*—a term that is used to categorize all the types of asbestos particles which are recorded during the analysis (such as fibers, bundles, clusters, and matrices).

4. Summary of Test Method

4.1 The sample is collected by vacuuming a known surface area with a standard 25 or 37 mm air sampling cassette using a plastic tube that is attached to the inlet orifice which acts as a nozzle. The sample is transferred from inside the cassette to a 50/50 alcohol/water solution and screened through a 1.0 by 1.0 mm screen. The fine dust is filtered onto a membrane filter and ashed in a muffle furnace. The ash is mixed with distilled water to a known volume. Aliquots of the suspension are then filtered through a membrane. A section of the membrane is prepared and transferred to a TEM grid using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using SAED and EDXA at a magnification dependent on the size range of asbestos structures present.

5. Significance and Use

5.1 This microvacuum sampling and indirect analysis method is used for the general testing of non-airborne dust samples for asbestos. It is used to assist in the evaluation of dust that may be found on surfaces in buildings, such as ceiling tiles, shelving, electrical components, duct work, carpet, etc. This test method provides an estimate of the mass concentration of asbestos in the dust reported as either the mass of asbestos per unit area or as the mass of asbestos per mass of sampled dust as derived from a quantitative TEM analysis.

5.1.1 This test method does not describe procedures or techniques required to evaluate the safety or habitability of buildings with asbestos-containing materials, or compliance with federal, state, or local regulations or statutes. It is the

[6] The boldface numbers refer to the list of references at the end of the test method.

[7] The non-asbestiform variations of the minerals indicated in 3.1.2 have different Chemical Abstract Service (CAS) numbers.

user's responsibility to make these determinations.

5.1.2 At present, a single direct relationship between asbestos-containing dust and potential human exposure does not exist. Accordingly, the user should consider these data in relationship to other available information in their evaluation.

5.2 This test method uses the definition *settleable particulate matter* found in Test Method D 1739 as the definition of dust. This definition accepts all particles small enough to pass through a 1 mm screen. Thus, a single, large asbestos-containing particle(s) (from the large end of the particle size distribution) disassembled during sample preparation may result in anomalously large asbestos concentration results in the TEM analyses of that sample. Conversely, failure to disaggregate large particles may result in anomalously low asbestos mass concentrations. It is, therefore, recommended that multiple independent samples be secured from the same area, and that a minimum of three samples be analyzed by the entire procedure.

6. Interferences

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

6.1.1 *Antigorite*.

6.1.2 *Palygorskite (Attapulgite)*.

6.1.3 *Halloysite*.

6.1.4 *Pyroxenes*.

6.1.5 *Sepiolite*.

6.1.6 *Vermiculite scrolls*.

6.1.7 *Fibrous talc*.

6.1.8 *Hornblende* and other amphiboles not listed in 5.1.3.

6.2 Collection of any dust particles greater than 1 mm in size in this test method may cause an interference and, therefore, should be avoided.

7. Apparatus

7.1 *Transmission Electron Microscope (TEM)*, an 80 to 120 kV TEM, capable of performing electron diffraction, with a fluorescent screen inscribed with calibrated gradations, is required. The TEM must be equipped with energy dispersive X-ray spectroscopy (EDXA) and it must have a scanning transmission electron microscopy (STEM) attachment or be capable of producing a spot size of less than 250 nm in diameter at crossover.

7.2 *Energy Dispersive X-ray System (EDXA)*.

7.3 *High Vacuum Carbon Evaporator*, with rotating stage.

7.4 *High Efficiency Particulate Air (HEPA)*, filtered negative flow hood.

7.5 *Exhaust or Fume Hood*.

7.6 *Particle-Free Water* (ASTM Type II, see Specification D 1193).

7.7 *Glass Beakers*, 50 mL.

7.8 *Glass Sample Containers*, with wide mouth screw cap (200 mL), or equivalent sealable container (height of the glass sample container should be approximately 13 cm high by 6 cm wide).

7.9 *Waterproof Markers*.

7.10 *Forceps (tweezers)*.

7.11 *Ultrasonic Bath*, table top model (100 W, approximate, see 22.5).

7.12 *Graduated Pipettes*, 1, 5, and 10 mL sizes, glass or plastic.

7.13 *Filter Funnel*, 25 mm or 47 mm (either glass or disposable). Filter funnel assemblies, either glass or disposable plastic, and using either a 25 mm or 47 mm diameter filter.

7.14 *Side Arm Filter Flask*, 1000 mL.

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

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

7.17 *Storage Containers*, for the 25 or 47 mm filters (for archiving).

7.18 *Glass Slides*.

7.19 *Scalpel Blades*.

7.20 *Cabinet-type Desiccator*, or low temperature drying oven.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[8] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 *Acetone*.

8.3 *Dimethylformamide (DMF)*.

8.4 *Chloroform*.

8.5 *1-methyl-2-pyrrolidone*.

8.6 *Glacial Acetic Acid*.

8.7 *Low Temperature Plasma Asher*.

8.8 *pH Paper*.

8.9 *Air Sampling Pump* (low volume personal-type pump).

8.10 *Rotameter*.

8.11 *Air Sampling Cassettes* (25 mm or 37 mm), containing 0.8 µm or smaller pore size MCE or PC filters.

8.12 *Cork Borer*, 7 mm.

8.13 *Non-Asbestos Mineral References*, as outlined in 6.1.

8.14 *Asbestos Standards*, as outlined in 3.1.2.

8.15 *Tygon*[9] *Tubing*, or equivalent.

8.16 *Vacuum Pump* (small), that can maintain a vacuum of approximately 92 kPa.

8.17 *Petri Dishes*, large, glass (approximately 90 mm in diameter).

[8] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[9] Tygon is a registered trademark of the DuPont Co.

8.18 *Stainless Steel or Aluminum Mesh Screen*, 30 to 40 mesh for Jaffe washer.

8.19 *Copper TEM Finder Grids*, 200 mesh.

8.20 *Carbon Evaporator Rods*.

8.21 *Lens Tissue*.

8.22 *Ashless Filter Paper*, 90 mm diameter, ASTM Class G (see Specification E 832).

8.23 *Reinforcement Rings*, gummed paper.

8.24 *Wash Bottles*, plastic.

8.25 *Reagent Alcohol*, HPLC grade (Fisher A995 or equivalent).

8.26 *Opening Mesh Screen*, plastic, 1.0 by 1.0 mm (SpectraMesh No. 146410 or equivalent).

8.27 *Static Neutralizer*.

8.28 *Muffle Furnace*, 480°C.

8.29 *Glazed Crucibles*.

8.30 *Jaffe Washer* **(4, 5)**.

8.31 *Diffraction Grating Replica*, with approximately 2160 lines/mm.

8.32 The following items are necessary if performing weight % calculations.

8.32.1 *Analytical Balance*, with readability of 0.01 mg or less. Exercise special care for the proper zeroing of the balance.

8.32.2 *Weighing Room*, with temperature and humidity control to allow weighing with an analytical balance to ±0.01 mg.

8.32.3 *Class M Weights*, for calibration of the analytical balance.

9. Sampling Procedure For Microvacuum Technique

9.1 For sampling asbestos-containing dust in either indoor or outdoor environments, commercially available cassettes must be used. Cassettes and sampling nozzles must be new and not previously used. Air monitoring cassettes containing 25 mm or 37 mm diameter mixed cellulose ester (MCE) or polycarbonate (PC) filter membranes with a pore size less than or equal to 0.8 µm are required. The number of samples collected depends upon the specific circumstances of the study.

9.2 Maintain a log of all pertinent sampling information and sampling locations.

9.3 Sampling pumps and flow indicators shall be calibrated using a certified standard apparatus or assembly (see Practice D 3195).

9.4 Record all calibration information **(6)**.

9.5 Perform a leak check of the sampling system at each sampling site by activating the pump with the closed sampling cassette in line. Any air flow shows that a leak is present that must be eliminated before initiating the sampling operation.

9.6 Attach the sampling cassette to the sampling pump at the outlet side of the cassette with plastic tubing (see 8.15). The plastic tubing shall be long enough that sample areas can be reached without interference from the sampling pump. Attach a clean, approximately 25.4 mm long, piece of plastic tubing (6.35 mm internal diameter) directly to the inlet orifice. Use this piece of tubing as the sampling nozzle. Cut the sampling end of the tubing at a 45° angle as illustrated in Fig. 1. The exact design of the nozzle is not critical as long as some vacuum break is provided to avoid simply pushing the dust around on the surface with the nozzle rather than vacuuming it into the cassette. The internal diameter of the nozzle and flow rate of the pump may vary as long as the air velocity within the body of the nozzle is 100 (±10) cm/s. This air velocity can be achieved with an internal sampling tube diameter of 6.35 mm (¼ in.) and a flow rate of 2 L/min.

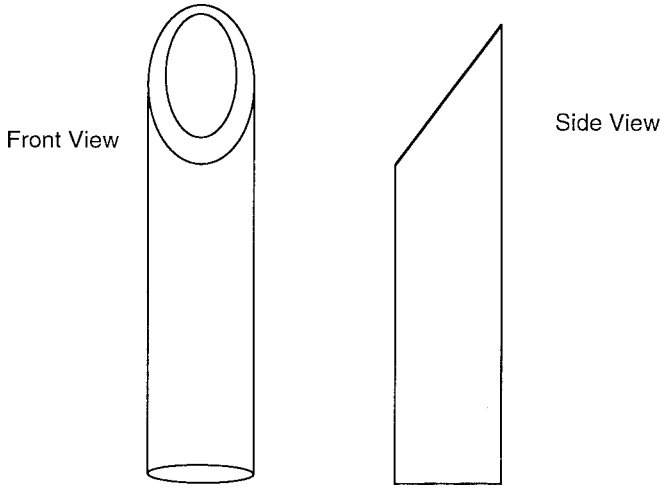

FIG. 1 Sampling End of Tubing

9.7 Delineate and measure the surface area of interest. A sample area of 100 cm^2 is vacuumed until there is no visible dust or particulate remaining. Perform a minimum of two orthogonal passes on the surface with a minimum of 2 min of sampling time. Avoid scraping or abrading the surface being sampled. (Do not sample any debris or dust particles greater than 1 mm in diameter (see 5.2)). Smaller or larger areas can be sampled, if needed. For example, some surfaces of interest may have a smaller area than 100 cm^2. Less dusty surfaces may require vacuuming of larger areas. Unlike air samples, the overloading of the cassettes with dust will not be a problem. As defined in 3.2.5, only dust shall be collected for this analysis.

9.8 At the end of the sample collection, invert the cassette so that the nozzle inlet faces up before shutting off the power to the pump. The nozzle is then sealed with a cassette end plug and the cassette/nozzle taped or appropriately packaged to prevent separation of the nozzle and cassette assembly. A second option is the removal of the nozzle from the cassette, then plugging of the cassette and shipment of the nozzle (also plugged at both ends) sealed in a separate closeable plastic bag. A third option is placing the nozzle inside the cassette for shipment. The nozzle is always saved to be rinsed because a significant percentage of the dust drawn from a lightly loaded surface may adhere to the inside walls of the tubing.

9.9 Check that all samples are clearly labeled, that all dust sampling information sheets are completed, and that all pertinent information has been enclosed, in accordance with laboratory quality control practices, before transfer of the samples to the laboratory. Include an unused cassette and nozzle as a field blank.

9.10 Wipe off the exterior surface of the cassettes with disposable wet towels or “baby wipes” prior to packaging for shipment.

10. Sample Shipment

10.1 Ship dust samples to an analytical laboratory in a

sealed container, but separate from any bulk or air samples. The cassettes must be tightly sealed and packed in a material free of fibers or dust to minimize the potential for contamination. Plastic bubble pack is probably the most appropriate material for this purpose.

11. Sample Preparation

11.1 Under a negative flow HEPA hood, carefully wet-wipe the exterior of the cassettes to remove any possible contamination before taking cassettes into a clean preparation area.

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

11.3 Initial specimen preparation 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 dust sample. Cleanliness of the preparation area hoods is measured by the cumulative process blank concentrations (see Section 12).

11.4 If a weight % determination is reported, pre-weigh the laboratory filter in accordance with this section, otherwise, proceed with 11.5.

11.4.1 Dry the laboratory filter under vacuum in a vacuum desiccator for at least 15 min.

11.4.2 Release the vacuum, remove the desiccator cover, and equilibrate the laboratory filters in the weighing room for at least 1 h.

11.4.3 Weigh the laboratory filter and record the filter tare weight, W1 (mg).

11.4.3.1 Zero the balance before each weighing.

11.4.3.2 Handle the filter with forceps.

11.4.3.3 Pass the filter over an antistatic radiation source. Repeat this step if the filter does not release easily from the forceps or if the filter attracts the balance pan.

11.4.4 Allow the laboratory filter to stand in the weighing room for an additional 8 to 16 h.

11.4.5 Reweigh the laboratory filter. If the second weight differs by more than 0.01 mg from the first tare weight (W1), discard the filter.

11.5 All sample preparation steps 11.5.1-11.5.6 shall take place in the dust preparation area inside a HEPA hood.

11.5.1 Remove the upper plug from the sample cassette and container and carefully introduce approximately 10 mL of a 50/50 mixture of particle-free water and reagent alcohol into the cassette using a plastic wash bottle. If the plugged nozzle was left attached to the cassette, then remove the plug and introduce the water/alcohol into the cassette through the tubing, and then remove the tubing if it is visibly clean.

11.5.2 Replace the upper plug or the sample cap, and lightly shake the dust suspension by hand for 3 s.

11.5.3 Remove the entire cap of the cassette and pour suspension through a 1.0 by 1.0 mm opening screen into a pre-cleaned 200 mL glass specimen bottle. All visible traces of the sample contained in the cassette shall be rinsed onto the screen with a plastic wash bottle containing the 50/50 mixture of water and alcohol. Repeat this procedure two additional times for a total of three washings. Next, rinse the nozzle two or three times through the screen into the specimen bottle with the 50/50 mixture of water and alcohol. Typically, the total amount of the 50/50 mixture used in the rinse is 50 to 75 mL. Remove the 1.0 by 1.0 mm screen and discard.

11.5.4 Use either a disposable plastic filtration unit or a glass filtering unit for filtration of the suspension. If a weight % determination is to be reported, use pre-weighed laboratory filters; see 11.4.

11.5.4.1 If a disposable plastic filtration unit is used, unwrap a new disposable plastic filter funnel unit (either 25 or 47 mm diameter) and remove the tape around the base of the funnel. Remove the funnel and discard the top filter supplied with the apparatus, while retaining 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. Wet it with a few mL of particle-free water and place an MCE or PC filter (≤0.2 µm pore size) 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 requires discard of that filter. After the filter has been seated properly, replace the funnel, and reseal it with the tape. Return the flask to atmospheric pressure.

11.5.4.2 If a glass filtration unit is used, place a 5 µm pore size MCE (backing filter) on the glass frit surface. Wet the filter with particle-free water, and place an MCE or PC filter (≤0.2 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. Before filtration of the sample suspension, prepare a blank filter by filtration of 50 mL of particle-free water. 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, then rub the surface with a clean paper towel soaked in a detergent solution. Repeat the cleaning operation, and then rinse several times in particle-free water.

11.5.5 Pour the suspension into the filter funnel. Rinse the beaker into the funnel at least three times, using distilled water.

11.5.6 Apply vacuum to the flask and draw the mixture through the filter.

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

11.6 If a weight % determination is reported, weigh the filter and solids using the method described in this section.

11.6.1 Place the filter into a low temperature drying oven (60°C) for 1 h.

11.6.2 Remove the filter and allow the filter to equilibrate in the weighing room for at least 1 h.

11.6.3 Weigh the filter and record the filter weight, W2 (mg).

11.6.3.1 Zero the balance before each weighing.

11.6.3.2 Handle the filter with forceps.

11.6.3.3 Pass the filter over an antistatic radiation source. Repeat this step if the filter does not release easily from the forceps or if the filter attracts the balance pan.

11.6.3.4 Use the same balance for weighing the filters before and after filtration of the suspension.

11.7 Place the filter or a measured portion of the filter into a glazed crucible and cover with an appropriate lid.

11.7.1 Pre-heat the muffle furnace to 480°C. Regulate this temperature at ±5°C throughout the ashing of the sample.

11.7.2 Place the crucible in the muffle furnace. Ash the sample for 12 h, or until the weight has stabilized.

11.7.3 Remove the crucible from the muffle furnace and allow it to cool.

11.8 Rinse the ash from the crucible into a 200 mL glass specimen bottle. Rinse all visible traces of the sample contained in the crucible into the specimen bottle using a plastic wash bottle containing particle-free water. This procedure is repeated two additional times for a total of three washings.

11.8.1 Bring the volume of the suspension up to 100 mL using distilled water.

11.8.2 Adjust the pH of the suspension to 3 to 4 by using a 10.0 % solution of acetic acid. Use pH paper for testing. Filter the suspension within 24 h to avoid problems associated with bacterial and fungal growth.

11.9 Use either a disposable plastic filtration unit or a glass filtering unit for filtration of portions of the suspension. The ability of an individual filtration unit to produce a uniformly loaded filter may be tested by filtration of a colored particulate suspension such as carbon black.

11.9.1 If a disposable plastic filtration unit is used then unwrap a new disposable plastic filter funnel unit (either 25 or 47 mm diameter) and remove the tape around the base of the funnel. Remove the funnel and discard the top filter supplied with the apparatus, while retaining 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. Wet it with a few mL of particle-free water and place an MCE or PC filter (≤0.2 µm pore size) 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 dictates discarding of that filter. After the filter has been seated properly, replace the funnel and reseal it with the tape. Return the flask to atmospheric pressure.

11.9.2 If a glass filtration unit is used, place a 5 µm pore size MCE (backing filter) on the glass frit surface. Wet the filter with particle-free water, and place an MCE or PC filter (≤0.2 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. Before filtration of each set of sample portions, prepare a blank filter by filtration of 50 mL of particle-free water. If portions of the same sample are filtered in order of increasing concentration, the glass filtration unit need not be washed between filtrations. 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, then rub the surface with a clean paper towel soaked in a detergent solution. Repeat the cleaning operation, and then rinse several times in particle-free water.

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

11.10 Briefly hand shake (3 s) the capped bottle with the sample suspension, then place it in a tabletop ultrasonic bath and sonicate for 15 min. Maintain the water level in the sonicator at the same height as the solution in the sample bottle. Carry out preparation steps 11.10-11.11.5 in a HEPA hood.

11.10.1 Calibrate the ultrasonic bath in accordance with 22.5. The ultrasonic bath must be operated at equilibrium temperature.

11.11 Estimate the amount of liquid to be withdrawn to produce an adequate filter preparation. Experience has shown that 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. Draw this portion from the suspension while it is in the sonicator. If after examination in the TEM, the smallest volume filtered (1.0 mL) yields an overloaded sample, perform additional serial dilutions of the suspension.

11.11.1 If serial dilutions are required, repeat step 11.10 before the serial dilution portion is taken. The recommended procedure for a serial dilution is to mix 10 mL of the sample solution with 90 mL of particle-free water in a clean sample bottle to obtain a 1:10 serial dilution. Follow good laboratory practices when performing serial dilutions.

11.11.2 Insert a new disposable pipette halfway into the sample suspension and withdraw a portion. Avoid pipetting any of the large floating or settled particles. Uncover the filter funnel and dispense the mixture from the pipette into the water in the funnel.

11.11.3 Apply vacuum to the flask and draw the mixture through the filter.

11.11.4 Discard the pipette.

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

11.11.6 In order to ensure that an optimally-loaded filter is obtained, it is recommended that filters be prepared from several different portions of the dust suspension. For this series of filters, it is recommended that the volume of each portion of the original suspension be a factor of five higher than the previous one. If the filters are prepared in order of increasing portion 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 filtrations.

11.11.7 There are many practical methods for drying MCE filters; the following are two examples that can be used: (*1*) dry MCE filters for at least 12 h (over desiccant) in an airtight cabinet-type desiccator; (*2*) to shorten the drying time (if desired), remove a plug of the damp filter and attach to a glass slide in accordance with 13.1.2 and 13.1.3. Place the slide with a filter plug or filter plugs (up to eight plugs can be attached to one slide) on a bed of desiccant, in the desiccator for 1 h.

11.11.8 PC filters do not require lengthy drying before