Designation: D 6056 – 96

Standard Test Method for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment by Transmission Electron Microscopy[1]

This standard is issued under the fixed designation D 6056; 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 the sampling methods and analysis techniques used to assess the airborne concentration and size distribution of single-crystal ceramic whiskers (SCCW), such as silicon carbide and silicon nitride, which may occur in and around the workplace where these materials are manufactured, processed, transported, or used. This test method is based on the filtration of a known quantity of air through a filter. The filter is subsequently evaluated with a transmission electron microscope (TEM) for the number of fibers meeting appropriately selected morphological and compositional criteria. This test method has the ability to distinguish among different types of fibers based on energy dispersive X-ray spectroscopy (EDS) analysis and selected area electron diffraction (SAED) analysis. This test method may be appropriate for other man-made mineral fibers (MMMF).

1.2 This test method is applicable to the quantitation of fibers on a collection filter that are greater than 0.5 μm in length, less than 3 μm in width, and have an aspect ratio equal to or greater than 5:1 **(1)**.[2] The data are directly convertible to a statement of concentration per unit volume of air sampled. This test method is limited by the amount of coincident interference particles.

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

1.4 *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[3]

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres[4]

D 4532 Test Method for Respirable Dust in Workplace Atmospheres[4]

D 6057 Test Method for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment by Phase Contrast Microscopy[4]

D 6058 Practice for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment[4]

D 6059 Test Method for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment by Scanning Electron Microscopy[4]

E 691 Practice for Conducting Interlaboratory Study to Determine the Precision of a Test Method[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *analytical sensitivity, n*—airborne fiber concentration represented by a single fiber counted in the TEM.

3.1.1.1 *Discussion*—Although the terms *fiber* and *whisker* are, for convenience, used interchangeably in this test method, whiskers is correctly applied only to single-crystal fibers whereas a fiber may be single- or poly-crystalline or may be noncrystalline.

3.1.2 *aspect ratio, n*—the ratio of the length of a fiber to its width.

3.1.3 *fiber, n*—*for the purpose of this test method,* an elongated particle having a minimum length of 0.5 μm, a width less than 3 μm, and an aspect ratio equal to or greater than 5:1.

3.1.4 *fibrous, adj*—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.

3.1.5 *man-made mineral fiber, n*—any inorganic fibrous material produced by chemical or physical processes.

3.1.6 *single-crystal ceramic whisker, n*— a man-made mineral fiber that has a single-crystal structure.

---

[1] This test method is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis and is the direct responsibility of Subcommittee D22.04 on Analysis of Workplace Atmospheres.

Current edition approved Dec. 10, 1996. Published February 1997.

[2] The boldface numbers in parentheses refer to a list of references at the end of this test method.

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

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

[5] *Annual Book of ASTM Standards*, Vol 14.02.

3.2 For definitions of other terms used in this test method, see Terminology D 1356.

4. Summary of Test Method

4.1 The sample is collected on a mixed cellulose ester (MCE) filter by drawing air, using a sampling pump, through an open-face 25-mm electrically conductive sampling cassette assembly **(2-4)**. A section of the filter is prepared and transferred to a TEM grid and the fibers are identified, sized, and counted at a screen magnification in the range from 8000 to 12 000× in the TEM in Section 11. Results are reported as a fiber concentration per unit volume of air and a fiber loading per unit area of filter. The airborne concentration is expressed as fibers per millilitre (f/mL) and the fiber loading is expressed as fibers per square millimetre (f/mm^2). Optionally, a supplementary low-magnification count in the range from 800 to 1200× may also be performed, using the criteria discussed in 11.1.5, to provide comparison with PCM data.

5. Significance and Use

5.1 The SCCW may be present in the workplace atmosphere where these materials are manufactured, processed, transported, or used. This test method can be used to monitor airborne concentrations of fibers in these environments. It may be employed as part of a personal or area monitoring strategy.

5.2 This test method is based on morphology, elemental composition, and crystal structure. The analysis technique has the ability to positively identify SCCW.

NOTE 1—This test method assumes that the analyst is familiar with the operation of TEM/EDS instrumentation and the interpretation of data obtained using these techniques.

5.3 This test method is applicable for the measurement of the total population of SCCW fibers including fibers with diameters $\leq$0.1 µm.

5.4 Results from the use of this test method shall be reported along with 95 % confidence limits for the samples being studied. Individual laboratories shall determine their intralaboratory coefficient of variation and use it for reporting 95 % confidence limits **(2,5,6)**.

6. Interferences

6.1 This test method has been designed to filter air for the determination of SCCW concentration. However, filtration of air also involves collection of extraneous particles and other fibers that may not be of interest. Extraneous particles may obscure fibers by overlay or overloading of the filter. This situation can be managed by regulating the air volume sampled and thus the filter loading. Fibers should appear separated from other particles to ensure an adequate opportunity for their recognition as separate entities in the TEM and accurate counting. Some coincident particulate agglomeration does occur even with these guidelines. Analyze an alternate filter with a reduced loading if the obscuring condition appears to exceed 15 % of the filter area **(7)**. Redeposition of a portion of an overloaded filter is permitted only in circumstances where an alternate filter is not available and cannot be obtained through resampling (see 10.1.12).

7. Apparatus and Reagents

7.1 *Sampling Cassette*—Use a 25-mm electrically conductive cassette assembly such as a three-piece cassette with an extension cowl or retainer ring containing a 0.45-µm pore size MCE filter and a support pad. Seal the cassette assembly with shrink tape. Reloading of used cassettes is not permitted.

7.2 *Personal Sampling Pump*—Use a portable battery-operated pump for personal sampling. Each pump must be capable of operating within the range from 0.5 to 4 L/min and continuously over the chosen sampling period **(2,3)**. The flow must be free from pulsation. All pumps shall be calibrated prior to use **(8)**.

7.3 *Area Sampling Pump*—Use a personal sampling pump or a non-portable high-volume pump for area sampling. Each pump shall be capable of operating within the range from 0.5 to 16 L/min and continuously over the chosen sampling period **(2,3)**. The flow shall be free from pulsation. All pumps shall be calibrated prior to use **(8)**.

7.4 *Vinyl* tubing or equivalent.

7.5 *Plasma Asher*, a low-temperature asher (LTA) is required to plasma-etch the collapsed MCE filter.

7.6 *Oxygen*, used as a bleed gas for plasma asher.

7.7 *Vacuum Evaporator*, for vapor deposition of conductive layers of carbon.

NOTE 2—Sputter coaters and carbonaceous fiber coaters are not appropriate.

7.8 *Specimen Grids*, copper 200-mesh TEM grids for mounting the specimen for TEM examination.

7.9 *Transmission Electron Microscope*— A TEM capable of operating using an accelerating voltage of at least 80 kV. The TEM must also be capable of performing EDS and SAED analyses. A light-element X-ray analyzer capable of detecting carbon, nitrogen, and oxygen is recommended. Use of a tilt-rotation holder as well as a double-tilt stage is also recommended. The TEM must have a fluorescent screen inscribed with calibrated gradations. It must be capable of producing a spot less than 250 nm in diameter at crossover under routine analytical conditions. Scanning transmission electron microscope (STEM) mode is allowed for this purpose.

7.10 *Sample Preparation Area*, consisting of either a clean room facility or a room containing a positive pressure HEPA-filtered hood.

7.11 *Tweezers*.

7.12 *Scalpel Blades*.

7.13 *Large Glass Petri Dishes* (approximately 90 mm in diameter).

7.14 *Jaffe Washer*.

7.15 *Lens Tissue*.

7.16 *MCE Filters*, 25 mm, 0.45 µm, and 0.22 µm.

7.17 *Funnel/Filtration Assembly*, 25 mm.

7.18 *Acetone*.

NOTE 3—**Precaution:** Acetone is a flammable liquid and requires precautions not to ignite it accidently.

7.19 *Specification D 1193 Type II Water* (particle free).

7.20 *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.[6] 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. Sample Collection

8.1 Collect samples of airborne SCCW on MCE filters using sampling cassettes and pumps in accordance with Section 7.

8.2 Remove the outlet plug from the sampling cassette and connect to a sampling pump by means of flexible, constriction proof tubing.

8.3 Perform a leak check of the sampling system by activating the pump with the closed cassette and rotameter (or other flow measurement device) in line. Any flow indicates a leak that must be eliminated before starting the sampling operation.

8.4 Remove the inlet plug from the sampling cassette to eliminate any vacuum that may have accumulated during the leak test; then remove the entire inlet cap.

8.5 Conduct personal and area sampling as follows:

8.5.1 For personal sampling, fasten the sampling cassette to the worker's lapel in the worker's breathing zone and orient face down. Adjust the calibrated flow rate to a value between 0.5 and 4 L/min **(2,3)**. Typically, a sampling rate between 0.5 and 2.5 L/min is selected **(4-7)**. Also see Test Method D 4532.

8.5.2 Place area samples on an extension rod facing down at a 45° angle. Adjust the calibrated flow rate to a value between 0.5 and 16 L/min **(2,3)**. Typically, a sampling rate between 1 and 10 L/min is selected **(1)**.

8.5.3 Set the sampling flow rate and time to produce an optimum fiber loading between 100 and 1300 f/mm^2 **(2-4)** . The time of sampling can be estimated by using the following equation:

$$t = \frac{(A_c)\,(F_L)}{(Q)\,(C_e)\,10^3} \qquad (1)$$

where:

A_c = active filter collection area (~385 mm^2 for 25-mm filter),[7]
t = time, min,
F_L = fiber loading, f/mm^2,
Q = sampling flow rate, L/min,
C_e = estimated concentration of SCCW, f/mL, and
10^3 = conversion factor.

8.5.4 At a minimum, check the flow rate before and after sampling. If the difference is greater than 10 % from the initial flow rate, the sample shall be rejected. Also see Test Method D 4532.

8.6 Carefully remove the cassette from the tubing at the end of the sampling period (ensure that the cassette is positioned upright before interrupting the pump flow). Replace the inlet cap and inlet and outlet plugs, and store the cassette.

NOTE 4—Deactivate the sampling pump prior to disconnecting the cassette from the tubing.

8.7 Submit at least one field blank (or a number equal to 10 % of the total samples, whichever is greater) for each set of samples. Remove the cap of the field blank briefly (approximately 30 s) at the sampling site, then replace it. The field blank is used to monitor field sampling procedures. Field blanks shall be representative of filters used in sample collection (for example, same filter lot number).

8.8 Submit at least one unused and unopened sealed blank which is used to monitor the supplies purchased as well as procedures used in the laboratory. The sealed blank shall be representative of filters used in sample collection (for example, same filter lot number).

9. Transport of Samples

9.1 Ship the samples in a rigid container with sufficient packing material to prevent jostling or damage. Care shall be taken to minimize vibrations and cassette movement.

NOTE 5—Do not use shipping material that may develop electrostatic forces or generate dust.

NOTE 6—Shipping containers for 25-mm sampling cassettes are commercially available and their use is recommended.

9.2 Include in the container a list of samples, their descriptions, and all other pertinent information.

10. Specimen Preparation

10.1 The objective of the specimen preparation technique is to produce a thin carbon film (sufficiently clear for the TEM analysis) containing the particles from the filter surface. This requires four separate preparation steps: (*1*) partially fuse or collapse the filter to obtain a more continuous surface for the evaporated carbon layer, (*2*) in a lowtemperature asher, lightly etch the filter surface to uncover any fibers that may have been covered in the collapsing step, (*3*) evaporate a thin carbon film over the collapsed and etched filter, and (*4*) dissolve the MCE filter and retain the carbon film with particles for TEM analysis. Procedures described as follows or other equivalent methods (for example, dimethyl formamide (DMF) procedure **(9)**) may be used to prepare samples.

10.1.1 Wipe the exterior of the cassettes with a damp cloth before taking them into the clean preparation area to minimize the possibility of contamination.

10.1.2 Perform specimen preparation in a clean area.

NOTE 7—At a minimum, the clean area must include a positive pressure HEPA-filtered hood.

10.1.3 Carefully cut a wedge of the filter area (for example, 25 %) with a curved, steel surgical blade using a rocking motion to prevent tearing.

NOTE 8—Use care not to disturb the particles on the filter surface.

10.1.4 Place the section, particle side up, on a clean microscope slide. Affix the filter section to the slide with a gummed page reinforcement or other suitable means. Label the slide with a glass scribing tool.

10.1.5 Place the slide in a petri dish which contains several paper filters soaked with acetone **(1)**. Cover the dish and wait for the filter to fuse and clear completely (typically, 2 to 4 min).

---

[6] *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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[7] The active collection area (A_c) should be measured periodically, especially if different types of cassettes are used.

10.1.6 Place the slide containing the collapsed filter into a low-temperature plasma asher, and etch the filter.

NOTE 9—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 conditions that shall be used. Insufficient etching will result in a failure to expose embedded fibers, and too much etching may result in loss of particles from the surface. It is recommended that the time for etching of a known weight of a collapsed filter be established and that the etching tare weight be calculated in terms of micrometres per second. The actual etching time used for a particular asher and operating conditions will then be set such that a ~1 to 2-µm layer (not more than 10 %) of collapsed surface will be removed **(1,10)**.

10.1.7 Place the slide inside the bell jar of a vacuum evaporator. Evaporate a section (1 mm in diameter by 8 mm in length) of graphite rod onto the etched filter. Remove the slide to a clean, dry, covered petri dish.

NOTE 10—Rotation of the sample at an angle (~45°) is recommended during the coating process.

10.1.8 Prepare a second petri dish as a Jaffe washer with the wicking substrate prepared from filter or lens paper **(1)**. The wicking substrate shall fit into the petri dish without touching the lid.

10.1.9 Identify the sample by labeling the petri dish or filter paper. In a fume hood, fill the petri dish with acetone until the wicking substrate is saturated. The level of acetone shall be just high enough to saturate the wicking substrate without creating puddles.

10.1.10 Remove a 3-mm square section of the carbon-coated filter from the glass slide using a surgical knife and tweezers. Carefully place the section of the filter, carbon side up, on the shiny side of a TEM grid. Cover the petri dish. Elevate one side of the petri dish by a few millimetres. This allows drops of condensed solvent vapors to form near the edge rather than in the center where they would drip onto the grid. Allow the sample to remain in the Jaffe washer until total dissolution of the MCE filter. Typically, a minimum of several hours is required to dissolve the MCE filter.

10.1.11 Three or more grids shall be prepared for each sample.

10.1.12 *Indirect Sample Preparation*—Resuspension of particulate matter collected on an overloaded filter and subsequent filtering onto another substrate may result in loss or breakup of the sample materials. Therefore, redeposition is permitted only in circumstances where an alternate filter is not available and cannot be obtained through resampling (for example, evaluation of a prototype procedure where the operational parameters cannot be duplicated). If indirect sample preparation procedures are employed, it must be clearly noted in the report. Furthermore, it must be clearly stated that results were obtained from the use of indirect sample preparation techniques and used only as an estimate of SCCW concentrations in the workplace environment. The following procedures are appropriate for this purpose.

10.1.12.1 Carefully remove the filter from the sampling cassette and cut a wedge (for example, one half or one quarter of the area of the original filter) as accurately as possible from the filter with a curved, steel surgical blade using a rocking motion to prevent tearing.

NOTE 11—Use care not to disturb the particles on the filter surface.

NOTE 12—The size of the wedge will depend on filter loading. If the sample is very heavily loaded, then a smaller wedge (for example, one eight or one sixteenth of the area of the original filter) may be more appropriate.

10.1.12.2 Place the section of filter into a 100-mL beaker.

10.1.12.3 Add approximately 80 mL of filtered Type II distilled water to the beaker.

10.1.12.4 Place the beaker into the ultrasonic bath. Sonicate for approximately 1 min.

10.1.12.5 Remove the section of filter and rinse it using filtered distilled water. The rinse shall be collected in the 100-mL beaker. Add enough distilled water to result in a 100-mL suspension.

10.1.12.6 Filter the suspension using a funnel through a 25-mm, 0.22-µm MCE filter using vacuum filtration techniques. Rinse the interior of the beaker into the funnel using filtered distilled water.

NOTE 13—It is recommended that disposable funnels be used to reduce the potential for contamination.

NOTE 14—Use of a 47-mm funnel/filter assembly is permissible provided the active filter area is accounted for in the calculations provided in 13.1.2.

NOTE 15—If the resuspended filter is too heavily loaded with particles to permit analysis, then reprepare the sample using a smaller portion of the original filter as discussed in 10.1.12.1.

NOTE 16—The MCE filters used for resuspension must have an average blank level less than 18 f/mm^2.

10.1.12.7 Remove the funnel from the vacuum system. Place the deposited filter in a desiccator for approximately 2 h to remove moisture.

10.1.12.8 Cut a wedge of the filter (for example, 25 %) with a curved, steel surgical blade and continue to follow the procedures outlined in 10.1.4-10.1.11.

NOTE 17—Account for the area of the filter used in the resuspension process in the equations provided in 13.1.2 when calculating the estimated airborne concentration. For example, if 25 % of the original filter area was redeposited onto a 25-mm filter, then a dilution factor of 4 is used in the calculations.

11. Analysis Method

11.1 The objective of this method is to determine the concentration of SCCW fibers per cubic millilitre of air sampled based on the number of SCCW fibers observed during the TEM analysis.

11.1.1 Place the specimen in the TEM and select an accelerating voltage of at least 80 kV.

11.1.2 To ensure representative analysis, one half of the sample area to be analyzed shall be analyzed on one sample grid preparation and the remaining half on the second sample grid preparation.

11.1.3 Examine specimens at low magnification (300 to 500×) for integrity and selection of grid openings for analysis.

11.1.3.1 Individual grid openings with greater than 5 % openings (holes) or covered with greater than 15 % particulate matter or obviously having nonuniform loading shall not be analyzed **(7)**. Reject the grid if:

(1) Less than 50 % of the grid openings covered by the replica are intact,

(2) The replica is doubled or folded in >50 % of its area,

(3) The replica is too dark because of incomplete dissolution of the filter, or

(4) Average filter loadings exceed 75 fibers/grid opening.

11.1.4 *Fiber Counting Rules* **(2,3)**:

11.1.4.1 Begin examination of the specimen at a calibrated screen magnification between 8000 and 12 000×. Perform fiber counting by starting at one end of a selected grid square opening (typically the top left corner). The initial traverse shall then be left to right and progressing in a straight line with slightly overlapping fields. At the end of the initial traverse, move the field of view down being careful to slightly overlap initial traverse and proceed in the opposite direction. Repeat this procedure until the grid opening is completed. The intent is to completely cover the grid square without having fibers missed or counted twice.

11.1.4.2 Count only ends of fibers that are greater than 0.5 µm in length, less than 3 µm in diameter, and have an aspect ratio equal or to greater than 5:1.

11.1.4.3 Count each fiber end within the grid square as one end, provided that the fiber is in accordance with 11.1.4.2.

11.1.4.4 Count visibly free ends that are in accordance with 11.1.4.2 and 11.1.4.3 when the fiber appears to be attached to another particle, regardless of the size of the other particle.

11.1.4.5 Estimate the length and width of each fiber using the inscribed markings on the TEM screen.

11.1.4.6 Count the free ends emanating from an agglomeration of fibers up to a maximum of 10 ends (5 fibers), provided that each segment is in accordance with 11.1.4.2 and 11.1.4.3.

NOTE 18—For agglomerates, the selection of up to 10 ends (5 fibers) is done on an arbitrary basis. However, for each fiber selected, the dimensions must be recorded. Furthermore, it must be noted on the count sheet that the fiber was part of an agglomerate.

NOTE 19—Figure 1 provides examples of fiber counts for possible fiber-end orientation.

11.1.4.7 Record the fiber counts and fiber dimensions on the count sheet. Record ND when no fibers are detected in the field.

11.1.4.8 Perform EDS on all fibers which meet the counting rules criteria. Identification criteria for EDS shall be based on the known chemistry of the fiber being processed, determined either from product formulation or by analysis of standard materials.

11.1.4.9 Indicate on the count sheet if an EDS spectrum was acquired but not recorded, along with any other appropriate information.

11.1.4.10 Document an EDS spectrum representative of each fiber type observed using a plotter or on a computer storage medium.

11.1.4.11 Record all appropriate information on the count sheet for each EDS spectrum that is plotted or stored.

11.1.4.12 The SAED may be used to identify individual fibers; for example, if identification and distinction between different SCCW fibers such as silicon carbide and silicon nitride is a requirement. Electron diffraction patterns and “*d*” values are to be compared with reference standards, known industry standards, or reference literature such as the Joint Committee for Powder Diffraction Standards (JCPDS) file.[8]

NOTE 20—It is recommended, but not required, that the electron microscope is equipped with a holder capable of obtaining zone axis diffraction patterns (either a double-tilt or rotation-tilt holder).

11.1.4.13 Record and store at least one SAED pattern for each sample. Record all appropriate identification documentation associated with the SAED pattern on the count sheet.

NOTE 21—The micrograph number and the negative of the recorded SAED patterns must be maintained in the laboratory’s quality assurance records.

NOTE 22—If SAED was attempted but no pattern was observed, so indicate on the count sheet.

11.1.4.14 Count a sufficient number of grid openings to obtain the analytical sensitivity desired. If 200 ends (100 fibers) are counted, stop analysis provided that a minimum of four grid openings has been analyzed.

NOTE 23—An analytical sensitivity of 0.01 f/mL is recommended.

NOTE 24—On samples with a heavy concentration of fibers, stop after 200 ends, but complete the grid opening being analyzed.

11.1.4.15 Divide the total ends count by 2 to yield fiber count.

11.1.5 In order to provide some correlation with PCM data, an additional count may be performed in the magnification range from 800 to 1200×. In this supplemental count (sometimes referred to as a PCM equivalent) only the subset of fibers whose lengths exceed 5 µm and whose widths exceed 0.2 µm are counted. Count a minimum of 40 grid squares or 200 fiber ends.

12. Quality Control

12.1 Monitoring the environment for airborne fibers requires the use of sensitive sampling and analysis procedures. The sensitivity of the analysis may be influenced by a variety of factors. These include the supplies used in the sampling and analysis operation, the performance of the sampling, the preparation of the sample grid from the filter, and the actual examination of the prepared grid in the TEM. Each of these unit operations must produce a product of defined quality if the analytical method is to produce a reliable and meaningful test result. Accordingly, a series of control checks and reference standards shall be performed along with the sample analysis as indicators that the materials used are adequate and that the operations are within acceptable limits. In this way, the quality of the data is defined and the results are of known value. These checks and tests also provide timely and specific warning of any problems that might develop within the sampling and analysis operations.

12.2 *Microscope Calibration*:

12.2.1 Make periodic performance checks of TEM magnification, SAED, and EDS systems.

12.2.2 All instrumentation shall be in calibration at the time the analysis is performed.

12.2.2.1 *Magnification Calibration*—The magnification calibration shall be done at the fluorescent screen. The TEM

---

[8] Inorganic Index to the Powder Diffraction File, Publication PD1S-21i, Published by the Joint Committee on Powder Diffraction Standards, 1601 Park Lane, Swarthmore, PA 19081.

shall be calibrated at the magnification used to measure the grid opening and also at the magnification used for fiber counting. This is performed with a cross-grating replica (for example, one containing at least 2160 lines/mm). Define a field of view on the fluorescent screen either by markings or physical boundaries. The field of view shall be measurable or previously inscribed with a scale or concentric circles (all scales should be metric). A logbook shall be maintained, and the dates of calibration and the values shall be recorded. The frequency of calibration depends on the past history of the particular microscope. After any maintenance of the microscope that involved adjustment of the power supplied to the lenses or the high-voltage system or the mechanical disassembly of the electron optical column apart from filament exchange, the magnification shall be recalibrated. Before the TEM calibration is performed, the analyst shall ensure that the cross-grating replica is placed at the same distance from the objective lens as the specimens. For instruments that incorporate a eucentric tilting specimen stage, all specimens and the cross-grating replica shall be placed at the eucentric position.

12.2.2.2 *Determination of Camera Constant*— The camera length of the TEM in SAED operating mode shall be calibrated before SAED patterns on unknown samples are observed. This can be achieved 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 SAED 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 one or two sharp rings are obtained on the superimposed SAED pattern. A thicker gold film would normally show multiple gold rings, but it will tend to mask weaker diffraction spots from the unknown fibrous particulate. An average camera constant using multiple gold rings can be determined. The camera constant is one half the diameter of the rings times the interplanar spacing of the ring being measured. For instruments that incorporate a eucentric tilting specimen stage, all specimens and the crossgrating replica shall be placed at the eucentric position.

12.2.2.3 The EDS system is calibrated conforming to the manufacturer's specifications or other accepted procedures. At a minimum, the calibration procedures should take into account the energy level and intensity.

12.3 *Blank Analysis*:

12.3.1 Analyze the field and sealed blanks before the field samples. The average of the blank samples must be less than 18 f/mm ^{2}. If a higher average value is obtained or if any single blank exceeds 54 f/mm^2, then the entire sampling and analytical procedure shall be examined carefully to locate and correct any source of the contamination.

12.3.2 Report the counts on each blank. Calculate the mean of the blank counts and subtract this value from each sample count before reporting the results.

12.3.3 Maintain as part of the laboratory quality assurance program a set of reference samples **(12)**. These samples shall consist of filter preparations including a range of loadings and background SCCW levels from a variety of sources including in-house or other laboratory field samples. The quality assurance officer shall maintain custody of the reference samples and shall supply each analyst with reference samples on a routine basis as part of the laboratory quality assurance program. The labels on the reference samples shall be changed periodically so that an analyst does not become too unduly familiar with the samples.

12.3.4 Estimate the laboratory intra- and inter-counter relative standard deviation from blind repeat counts expressed as fiber loading (f/mm^2) on reference samples. Obtain separate values of relative standard deviation for each sample matrix analyzed in each of the following ranges: from 5 to 20 fibers in 10 grid openings, from >20 to 50 fibers in 10 grid openings, from >50 to 100 fibers in 10 grid openings, and 100 fibers in less than 10 grid openings. Maintain control charts for each of these data files **(2,12)**. Calculate S_R as one half of the pooled, intra-counter relative standard deviation **(12)**.

NOTE 25—The intralaboratory intra-counter relative standard deviation (S_R) shall be less than 0.20 for a laboratory to be considered proficient in the method **(2,5,6)**.

12.4 *Replicate and Duplicate Analyses*:

12.4.1 The primary method for the precision and accuracy of an individual analyst is through the use of replicate and duplicate analyses. A replicate analysis is a repeat analysis of the same prepared sample, performed by the same analyst under the identical analytical conditions as the original analysis. The frequency of this analysis is one replicate for every 100 samples analyzed. A duplicate analysis is performed in a similar manner to the replicate analysis, but with a different analyst. The frequency of this analysis is one duplicate for every 10 samples analyzed.

12.4.2 *Replicate Analyses*:

12.4.2.1 Document the precision of each analyst using replicate fiber counts.

12.4.2.2 The conformance expectation for the replicate analysis is that the fiber loading (f/mm^2) from the original and the replicate analyses will fall within the following control limits:

$$|\sqrt{A_1} - \sqrt{A_2}| \leq 2.77 \left(\frac{\sqrt{A_1} + \sqrt{A_2}}{2} \right) S_R \qquad (2)$$

where:

A_1 = original estimate of fiber loading,

A_2 = replicate estimate of fiber loading, and

S_R = one-half of the pooled, intra-counter relative standard deviation **(12)**.

Control limits are established from historical data. If the original and the replicate estimate falls outside the acceptance range, the sample is reexamined to determine the cause of the count variation. If the reexamination shows the analyst may be in error due to questionable ability, the analyst may not be permitted to examine unknown samples but must recount five reference samples. Upon acceptable performance of the analyses, the analyst may again examine unknown samples, but the frequency of replicate analyses is increased to one in every five samples for the next 100 samples, or until such replicate analyses meet the conformance expectations.

12.4.2.3 If the analyst fails the replicate test, all samples in the sample set shall be recounted and the new counts compared with the original count. All rejected counts will be discarded and the samples reanalyzed.

12.4.3 *Duplicate Analyses*:

12.4.3.1 One method of determining the accuracy of an analyst is to duplicate the analysis. A duplicate analysis is performed in a similar manner to the replicate analysis, but with a different analyst. The second analyst is randomly selected to perform the analysis. Conformance expectations are similar to replicate analyses, or:

$$|\sqrt{A_1} - \sqrt{A_2}| \leq 2.77 \left(\frac{\sqrt{A_1} + \sqrt{A_2}}{2} \right) S_R \tag{3}$$

where:

A_1 = original estimate of fiber loading,

A_2 = duplicate estimate of fiber loading, and

S_R = one-half of the pooled, intercounter relative standard deviation **(12)**.

12.4.3.2 If the duplicate analysis fails to conform with the acceptance criteria, both analysts must reanalyze the samples to determine the cause of the variation. If the reexamination shows that one or both of the analysts may be in error due to questionable ability, the analyst(s) shall perform verified analyses and meet the conformance expectations before being allowed to analyze unknown samples.

12.5 Each new analyst shall be instructed in the operation of the instrumentation discussed in this test method.

NOTE 26—To ensure good reproducibility, all laboratories engaged in SCCW counting should participate with other laboratories in the exchange of field samples to compare performance of the analysts (also see Practice E 691 for guidelines).

12.6 *TEM Grid Measurement*—Random 200-mesh copper grids shall be measured on a routine basis to document the average area of a typical grid opening. The measurement can be performed by placing a grid on a glass slide and examining it under the PCM at a magnification of approximately 400×. Use a calibrated graticule to measure the field length and width. From the data, calculate the field area for an average grid opening. Optical microscopy utilizing automated procedures may be used providing instrument calibration can be verified. Measurements can also be noted from the TEM at a properly calibrated low magnification. Do not use grids whose variability (as determined by the standard deviation of the measurements) is greater than 6 % of the mean value.

NOTE 27—Purchase and use of pre-certified TEM sample grids is permissible.

12.7 Appropriate logs or records must be maintained by the analytical laboratory verifying that it is in compliance with the quality assurance procedures.

13. Calculations

13.1 The following information must be reported for each sample analyzed by TEM: number of SCCW counted, area analyzed (mm ^{2}), volume of air sampled in litres (L), SCCW loading (f/mm^2), SCCW airborne fiber concentration (f/mL), and analytical sensitivity (f/mL). The calculations used to obtain SCCW loading and airborne SCCW concentration are as follows:

13.1.1 *Calculation for Direct Preparation*:

13.1.1.1 *SCCW Counts (N)*:

$$N = \frac{\text{number SCCW ends counted}}{2} \tag{4}$$

13.1.1.2 *SCCW Fiber Loading (F_L)*:

$$F_L = \left(\frac{N}{\text{area analyzed}} \right)_{\text{SCCW Sample}} - \left(\frac{N}{\text{area analyzed}} \right)_{\text{Blank}} \tag{5}$$

13.1.1.3 *SCCW Airborne Concentration (C)*:

$$C = F_L \times \frac{\text{active filter area}}{\text{volume of air sampled } (L) \times 10^3} \tag{6}$$

13.1.1.4 *Analytical Sensitivity (A_s)*:

$$A_s = \frac{1 \text{ SCCW}}{\text{area analyzed}} \times \frac{\text{active filter area}}{\text{volume of air sampled } (L) \times 10^3} \tag{7}$$

13.1.2 *Calculations for Indirect Preparation*:

13.1.2.1 *SCCW Counts (N)*:

$$N = \frac{\text{number SCCW ends counted}}{2} \tag{8}$$

13.1.2.2 *SCCW Loading (F_L)*:

$$F_L = \frac{\left(\frac{N}{\text{area analyzed}} \times \text{ active filter area} \times \text{dilution} \right)_{\text{redeposited area}} - \left(\frac{N}{\text{area analyzed}} \times \text{active filter area} \right)_{\text{blank}}}{\text{active filter area of collection filter}} \tag{9}$$

13.1.2.3 *SCCW Airborne Concentration (C)*:

$$C = \frac{\left(\frac{N}{\text{area analyzed}} \times \text{active filter area} \times \text{dilution} \right)_{\text{redeposited area}} - \left(\frac{N}{\text{area analyzed}} \times \text{active filter area} \right)_{\text{blank}}}{\text{volume of air sampled } (L) \times 10^3} \tag{10}$$

13.1.2.4 *Analytical Sensitivity (A_s)*:

$$A_s = \frac{1 \text{ SCCW}}{\text{area analyzed}} \times \frac{\text{active redeposited filter area}}{\text{volume of air sampled } (L) \times 10^3} \times \text{ dilution factor} \tag{11}$$

14. Precision and Bias

14.1 The precision of the procedure described in this test method for measuring the concentration of single-crystal ceramic whiskers is being determined.

14.2 Since there is no accepted reference material suitable for determining the bias using the procedure described in this test method for measuring the concentration of single-crystal ceramic whiskers, bias has not been determined.

15. Keywords

15.1 air monitors; sampling and analysis; silicon-carbide whiskers; single-crystal ceramic whiskers; transmission electron microscopy; workplace atmosphere

REFERENCES

(**1**) USEPA, Asbestos-Containing Materials in Schools; Final Rule and Notice, Federal Register 52 (210), Appendix A to Subpart E, Oct. 30, 1987, pp. 41857–41884.

(**2**) Baron, P., "Fibers, Method 7400, Issue No. 2:8-15-94," *NIOSH Manual of Analytical Methods*, 4th ed., P. M. Eller, ed., U.S. Department of Health and Human Services, DHHS (NIOSH) Publication No. 94-113, Cincinnati, OH.

(**3**) Baron, P., "Asbestos Fibers, Method 7402, Issue No. 2:8-15-94," *NIOSH Manual of Analytical Methods*, 4th ed., P. M. Eller, ed., U.S. Department of Health and Human Services, DHHS (NIOSH) Publication No. 94-113, Cincinnati, OH.

(**4**) OSHA Reference Method ID-160, Microscopy Branch, Salt Lake City Analytical Laboratory, Occupational Safety and Health Administration, U.S. Department of Labor, Salt Lake City, UT 84115-0200, revised August 1990.

(**5**) Leidel, N. A., Bayer, S. G., Zumwalde, R. D., and Busch, K. A., "USPHS/NIOSH Membrane Filter Method for Evaluating Airborne Asbestos Fibers," U.S. Department of Health, Education, and Welfare, (NIOSH) 79-127, 1979.

(**6**) *NIOSH Manual of Analytical Methods*, 2nd ed., Vol 1, P&CAM 239, U.S. Department of Health, Education, and Welfare, (NIOSH) 77-157-A, 1977.

(**7**) Peck, A. S., Serocki, J. J., and Dicker, L. C., "Sample Density and the Quantitative Capabilities of PCM Analysis for Measurement of Airborne Asbestos," *Am. Ind. Hyg Assoc. J.*, Vol 47, April 1986, pp. 232–233.

(**8**) *OSHA Technical Manual*, Occupational Safety and Health Administration, U.S. Department of Labor, Washington, DC 20210, OSHA Instruction CPL 2-2.20B, Directorate of Technical Support, Feb. 5, 1990, pp. 1–8 to 1–11.

(**9**) Burdett, G. J., and Rood, A. P., "Membrane Filter, Direct Transfer Technique for the Analysis of Asbestos Fibers or Other Inorganic Particles by TEM," *Environ. Sci. Tech.*, 17, 1983, pp. 643–648.

(**10**) *NVLAP Program Handbook, Airborne Asbestos Analysis, NISTIR 89-4137*, U.S. Department of Commerce, National Institute of Standards and Technology, August 1989.

(**11**) "Reference Methods for Measuring Airborne Man-Made Mineral Fibres (MMMF)," WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF, World Health Organization, Copenhagen, 1985.

(**12**) Abell, M. T., Shulman, S. A., and Baron, P. A., "The Quality of Fiber Count Data," *Appl. Ind. Hyg.*, Vol 4, No. 11, November 1989, pp. 273–285.