SUBJECT	DATE	PAGE	ITEM NO.
Transmission Electron Microscope Method for Identifying and Quantitating Asbestos in Non-Friable Organically Bound Bulk Samples	11/22/06	1 of 23	198.4

1. **Introduction.** This is a revision to the method for the analysis of asbestos in non-friable organically bound (NOB) bulk materials.

1.1. **Background.** Vinyl asbestos tiles (VAT) have been categorized by NESHAP (1990) as a Category I non-friable asbestos-containing material. As such, VAT's potential to release fibers under certain conditions was recognized. Recent studies reinforce this potential for releasability (McDonald 1991, Brzezowski 1990, Marxhausen and Shaffer 1991). VATs present one of the most difficult challenges to laboratories that analyze bulk samples for asbestos. Fiber sizes used in manufacture were often the "shorts" that were too small for other applications (Rajhans and Sullivan 1981, Cossette and Delvaux 1979). These were often further comminuted during mixing (Mullin 1988) so that many of the fibers were below the resolution capability of the light microscope. The addition of organic matrix materials, usually vinyl or asphalt, compounded the detection problem by coating these small fibers with opaque matter. Finally, mechanical abrasion during sample preparation can further reduce fiber size and inhibit detection.

1.2. **Objective**. Transmission electron microscopy (TEM) provides the most reliable method for detecting and quantitating these fibers in NOBs. This method provides specific requirements for the analysis of NOBs by TEM, including analytical protocols, quality control, calibrations, and recordkeeping.

1.3. **Definitions**

1.3.1. **Asbestos**. "Asbestos" refers to the asbestiform varieties of: chrysotile (serpentine); crocidolite (riebeckite); amosite (cummingtonite-grunerite); anthophyllite; tremolite; and actinolite (AHERA, 1987).

1.3.2. **Asbestos-Containing Materials**. "Asbestos-containing materials" (ACM) means any material or product that contains more than 1 percent asbestos (AHERA, 1987; NESHAP, 1990).

1.3.3. **Friable**. "Friable" materials are those materials that, when dry, may be crumbled, pulverized, or reduced to powder by hand pressure, and includes previously nonfriable material after such previously nonfriable material becomes damaged to the extent that when dry it may be crumbled, pulverized, or reduced to powder by hand pressure (AHERA, 1987).

1.3.4. **Vinyl Asbestos Tile**. This term (VAT) has been widely used in the asbestos analysis and abatement industry to refer to a variety of flooring products such as vinyl or asphalt asbestos floor tiles and resilient floor coverings.

1.3.5. **Non-Friable Organically Bound Materials**. This term (NOB) refers to a wide variety non-friable building materials embedded in flexible-to-rigid asphalt or vinyl matrices. This includes VAT, mastic, asphalt shingles, roofing materials, paint chips, caulking and glazing, etc.

1.4. **Principles**. A variety of methods have been used with varying success in identifying and quantitating asbestos in bulk materials. In addition to TEM (summarized

in the next paragraph) polarized-light microscopy, scanning electron microscopy, and x-ray diffraction are also methods used to analyze asbestos in bulk samples. Each of these methods has serious shortcomings for detecting asbestos in NOBs.

TEM's extremely short wavelength of 80-100 keV electrons coupled with simple image presentation yields resolvable images of even the smallest (~0.02 μm) asbestos fibrils. In fact, the then-theoretical 020 (4.5 Å) and 002 (7.3 Å) spacings of the scrolled fibrils were dramatically revealed in cross sections more than 30 years ago by TEM (Yada 1969, 1971). Furthermore, identification of chrysotile or amphibole crystalline structure can be consistently determined via the electron-diffraction capabilities of modern TEMs. Finally, the five amphibole types can be differentiated based on their elemental composition when energy-dispersive x-ray analysis (EDX) is utilized. The major drawback to TEM is accurate and reproducible subsampling; since only ~10^{-6} of the original sample is viewed via TEM, extreme precautions must be taken to prevent potentially enormous subsampling bias.

2. **Application**. Friable materials must be analyzed by one of the point-counting methods (ELAP Item 198.1) while NOBs must be analyzed by one of the gravimetric matrix reduction methods, either the PLM method (ELAP Item 198.6) or the TEM method described herein. This TEM method is the **only** method that can be used to report true negative results from NOB samples to clients.

3. **Equipment and Supplies** The following items must be available for sample preparation and analysis in laboratories that analyze bulk samples by TEM:

3.1. HEPA-ventilated, negative-pressure sample preparation work area. This can be a laminar-flow safety cabinet or a similar enclosure that draws all air from the enclosure through a HEPA filter. This should minimize cross contamination and maintain a safe work environment. A flow rate of at least 75 fpm shall be maintained at the opening.

3.2. Low-power (10-45X) stereobinocular microscope with external light source for gross examination.

3.3. Forceps, dissecting needles, probes, scalpel or razor blades, etc., for manipulating bulk sample.

3.4. Homogenization equipment:

3.4.1. Mortar and pestle

3.4.2. At least one of the following:

3.4.2.1. Mini-blender (approximately 30 mL capacity)

3.4.2.2. Liquid-nitrogen mill

3.4.2.3. Wiley mill

3.5. Centrifuge.

3.6. Filtration apparatus for polycarbonate filters.

3.6.1. 0.4-µm pore polycarbonate filters.

3.6.2. Petri dishes and covers.

3.7. Muffle furnace capable of sustained operation at 500°C.

3.7.1. Crucibles (bottom and lid) that can withstand 500°C.

3.7.2. Instrument or materials capable of calibrating muffle furnace at 480°C:

3.7.2.1. High-temperature thermometer with range to at least 500°C and with readable subdivisions of 5°C or less ***or***

3.7.2.2. Melting-point solids with capability of differentiating 5°C differences between 400°C and 500°C ***or***

3.7.2.3. Independent potentiometer capable of differentiating 5°C differences between 400°C and 500°C.

3.8. Concentrated HCl, reagent grade.

3.9. Filtered (0.1-µm) ethanol.

3.10. Heat lamp, slide warmer or drying oven.

3.11. Ultrasonic bath.

3.12. Filtered (0.1-µm) distilled water.

3.13. References.

3.13.1. Copy of Chatfield (1991) method.

3.13.2. Copy of non-mandatory analytical section of AHERA (1987).

3.13.3. Textbook or reference book on mineralogy or crystallography, e.g., McCrone 1980, McCrone 1988, Deer, Howie and Zussman, 1966.

3.13.4. References required as part of AHERA analysis (e.g., verified asbestos analysis, spot-size measurement, k-factor determination).

3.13.5 **Reference Library**: The laboratory must develop its own collection of reference materials and information on all asbestos types and other fiber types that commonly interfere with asbestos analysis by TEM. This information must be readily available to all TEM analysts. Electron micrographs (at routine magnification) of images and zone-axis ED patterns (at routine camera length) of single fibrils must be collected for all asbestos types with the laboratory's in-house TEM. From these electron micrographs, analysts should be able to predict the orientation of ED layer lines relative to the orientation of the fiber at normal TEM operating conditions. Photographic enlargements of these ED patterns should have the characteristic diffraction points and fiber axis orientations labeled. Additionally, hard-copy EDX spectra of each of the asbestos types must be produced using the in-house TEM/EDX system. Using the in-house TEM/EDX system, spectra must be collected

from six different fibers of each of the asbestos types. On the basis of these spectra, ranges of ratios (FWHM) of critical cations for each of the asbestos types relative to silicon must be calculated. For example, the ranges of Na:Si, Fe:Si, and Mg:Si would have to be calculated for crocidolite and be available to the TEM analyst.

At least four asbestos "look-alikes" must be on separate in-laboratory TEM grids. From these, the laboratory must have produced electron micrographs of images, electron diffraction patterns, and EDX spectra. The criterion or criteria that differentiate each of these from asbestos must be highlighted.

3.13.6. **Zone-Axis References**: The laboratory must have tables or software that will allow determination of specific zone-axis measurements for each asbestos type.

3.13.7. **Darkroom Supplies**: If the laboratory is using photographic methods to produce images and ED patterns, supplies must be on hand to process these images.

3.14. Reference materials.

3.14.1. National Institute of Standards and Technology (NIST) SRMs 1866a and 1867:

3.14.1.1. Chrysotile.

3.14.1.2. Amosite (grunerite).

3.14.1.3. Crocidolite (riebeckite).

3.14.1.4. Fibrous glass.

3.14.1.5. Anthophyllite.

3.14.1.6. Tremolite.

3.14.1.7. Actinolite.

3.14.2. Verified quantitative standards. All laboratories must have at least 10 different VAT specimens that have been analyzed by an ELAP-certified TEM laboratory (own lab or outside lab) using the quantitative method outlined herein. Raw gravimetric data and calculations used to determine percentages of each component (organic, carbonate, insoluble inorganic and asbestos) must be provided by the outside laboratories and kept on file. Each of these reference specimens should initially exceed 50 cm^2 in area.

Sections of these VATs must be submitted blindly as routine samples as outlined in Section 8.2.3 to determine the accuracy and precision of the laboratory's current analytical capabilities.

3.14.2.1. Negative (non-ACM) standards. At least two negative standards must be kept. While one of these may contain asbestos at a concentration of less than 1%, the other must be free of any asbestos for use in monitoring contamination (Section 8.3.2).

3.14.2.2. Positive (ACM) standards (low concentration). At least two positive standards with concentrations less than 10% must be kept.

3.14.2.3. Positive (ACM) standards (high concentration). At least two positive standards with concentrations greater than 10% must be kept.

3.14.2.4. At least four other different standards must be kept.

3.14.3. Permanent mount of NIST amosite in n_D=1.680.

3.15. Microscope slides.

3.16. Cover slips.

3.17. Refractive index liquids:

3.17.1. n_D=1.550 high dispersion.

3.17.2. n_D=1.605 high dispersion.

3.17.3. n_D=1.630 high dispersion.

3.17.4. n_D=1.680.

3.17.5. n_D=1.700.

3.17.6. Series of n_D=1.49 to 1.72 in intervals less than or equal to 0.005. This full series is required because of the range of refractive indices exhibited by the different asbestos types in both their natural and altered (heated or acid-stressed) states. High-dispersion liquids may be substituted in the 1.49 to 1.63 range.

3.17.7. Calibration accessory for measuring refractive indices of refractive index liquids. These can be calibrated solids, e.g., glasses, or a refractometer capable of an accuracy of ±0.004.

3.17.8. Laboratory thermometer with range of 0° to 50° C and readability of ±1° C.

3.18. Marker for labeling slides.

3.19. Polarizing-light microscope equipped with the following:

3.19.1. Substage polarizer.

3.19.2. Analyzer capable of producing a completely black field when privileged direction is oriented perpendicular to that of the substage polarizer. Either the polarizer or analyzer must be rotatable so that polars can be slightly uncrossed when necessary.

3.19.3. Port @ 45° to analyzer for wave retardation plate.

3.19.4. 550 nm (first-order red) compensator plate.

3.19.5. 360° graduated (in 1° increments) rotating stage.

3.19.6. Illuminator and adjustable diaphragm.

3.19.7. The following objective lenses:

3.19.7.1. Dispersion-staining objective capable of central stop illumination with magnification of approximately 10X (optional).

3.19.7.2. Low-magnification objective (3.2 to 10X).

3.19.7.3. High-magnification, dry objective (30 to 50X).

3.19.8. Eyepiece(s) of at least 8X magnification containing a fixed cross-hair.

3.19.8.1. Chalkley point-count reticle (optional).

3.19.9. Focusable condenser with centerable iris diaphragm capable of completely eclipsing the back-focal-plane image of the central stop.

3.20. Beam balance with readability of 1 mg or less. (e.g., Fisher Model 711).

3.21. Transmission electron microscope that must meet the specifications outlined in the AHERA method for airborne asbestos analysis (EPA 40 CFR 763 - Appendix A to Subpart 3, October 30, 1987 - **Non**-mandatory Method).

3.21.1 Specimen holder with at minimum a single-tilt capability through at least $\pm 25^{o}$.

3.21.2 Calibration lines on the TEM's phosphor screen must be drawn in at least two mutually perpendicular directions or drawn as calibrated-diameter circles. A single calibration line is acceptable only if the specimen holder or image is capable of continuous rotation during analysis without affecting magnification calibration.

3.22. Micropipette capable of dispensing 2-5 µL aliquots.

3.23. Disposable plastic beakers, approximately 25-50 mL capacity.

3.24. Analysis sheet (see Example 1 at end of Item 198.4) with space for the following entries:

3.24.1. Analyst's signature.

3.24.2. Date of analysis.

3.24.3. Sample identification number.

3.24.4. Gross description of bulk sample (color, homogeneity, texture) and tentative identification of fibers by stereobinocular microscope.

3.24.5. Type of homogenization (if any).

3.24.6. Matrix reduction. This should include ashing and acid steps used and amount of matrix removed during each step to the nearest mg.

3.24.6.1. Mass of original subsample.

3.24.6.2. Mass of post-ashed subsample.

3.24.6.3. Mass of post-acid subsample.

3.24.7. Entries for each asbestos type identified:

3.24.7.1. Morphology.

3.24.7.2. Electron diffraction data. This would include the negative or file number for each collected electron diffraction pattern and an identification of the pattern measured (chrysotile, amphibole, etc.).

3.24.7.3. Elemental composition from energy-dispersive x-ray spectrometer.

3.24.8. Space for estimation of asbestos percentages from both grids.

3.24.9. Space for results of PLM observation of residue.

3.24.10. Final results including:

3.24.10.1. Type(s) and percentage(s) of each asbestos type detected.

3.24.10.2. Total percentage of asbestos.

3.24.10.3. Percentage of non-asbestos insoluble inorganic fraction.

3.24.10.4. Percentage of soluble inorganic fraction.

3.24.10.5. Percentage of organic fraction.

4. **Sample Preparation**. Careful sample preparation is a critical step in the analysis of NOBs. Detection of asbestos fibers used in VATs is often extremely difficult because of the small fibers used during manufacture, their subsequent mixing and coating with organic matrix (vinyl, asphalt, etc.), and potential comminution during sample preparation. In general, the more material that can be removed and tracked gravimetrically, the fewer interferences will remain and the results should be more accurate and reliable. The steps outlined below are based on the Chatfield (1991) method and must be followed to maximize detection and accurate quantitation of asbestos. All mass determinations must be made to the nearest 1 mg or less. Filter residue prepared for PLM analysis of NOBs according to ELAP protocol (Item 198.6, Section 4.1 through 4.3) may be used for TEM grid preparation so that steps in Sections 4.1 through 4.4 do not have to be repeated.

The United States Environmental Protection Agency has clarified how bulk samples that contain multiple layers are to be analyzed and reported (U.S.E.P.A., 1995; U.S.E.P.A., 1994a; U.S.E.P.A., 1994b). Layered samples should be handled according to these guidelines.

4.1. **Preliminary Examination.** Each sample must be examined in its entirety by stereobinocular microscopy. (Preliminary PLM analysis is not mandatory as the NOB matrix materials will likely dissolve in RI liquid, making it impossible to measure fiber RI and to accurately measure asbestos quantity). This mandatory stereobinocular microscopical examination will serve three purposes.

4.1.1. Determine the existence of different materials or layers: Each discrete material must be prepared and analyzed separately.

4.1.2. Determine the overall homogeneity of the material: If the material is homogeneous, subsampling of sections for preparation and analysis can be at random.

4.1.3. Search for protruding fibers: Fibers protruding from the matrix may be removed for PLM analysis. These fibers must be matrix-free because dissolution of NOB matrix in RI liquids will change the liquid's RI and lead to incorrect measurements of fiber RI.

If the protruding fiber(s) is identified as asbestos, the laboratory may notify their client that the NOB contains asbestos at an *unquantified percentage* as detailed in 6.3.2.1 and that full gravimetric reduction will be necessary to determine a specific percentage. *This step cannot be used to report that the sample is an ACM or a non-ACM.*

The identification of asbestos by PLM in this step must be noted on the analysis sheet. If full gravimetric reduction and TEM analysis of this sample as required in this Item (198.4) yields 1% or less asbestos, the report to the client will state that the results are *Conflicting*, as detailed in 6.3.2.3.

4.2. **Preliminary Preparation**. All extraneous materials (mastic, wax, polish) should be removed before preparation is initiated. Mastic must be considered a separate sample and must be removed for independent analysis. For a sample with distinctly different layers that are separable, each layer must be prepared, analyzed, and reported separately (U.S.E.P.A 1994a, 1994b).

4.3. **Organic Reduction**. Shave off approximately 100-500 mg NOB into a tared crucible and weigh. The mass of the subsample must be at least 200 times greater than the readability of the laboratory's analytical balance. Place in muffle furnace at 480°C until mass stabilizes (1-12 hr). Cool to stable mass in a desiccator, reweigh and calculate percent organic loss.

4.4. **Acid Digestion**. Grind residue in crucible with 0.5 mL 0.1-µm-filtered distilled water and add 2-5 mL concentrated HCl. After 15 minutes, dilute with 0.1-µm-filtered distilled water and pour into filtration apparatus holding a tared 0.4-µm polycarbonate filter and apply vacuum. Rinse the crucible a second time and pour this into the filtration apparatus and rinse down the sides of the apparatus. When filtration is complete, transfer filter and residue carefully to a clean, tared plastic petri dish and allow filter to dry to stable mass under a heat lamp. Weigh filter and petri dish and calculate percent mineral carbonate loss. *If* the residue mass is less than 1% of the subsample's original mass, analysis may be terminated and the sample reported as a non-ACM (Section 6.3.2.1).

4.5. **Grid Preparation**. At least two grid preparations must be made from the residue on the filter. Excise a section (50-200 mm^2, depending on residue thickness) of the polycarbonate filter and transfer to a disposable plastic beaker. Add approximately 5 mL of filtered ethanol and treat in ultrasonic bath for one minute. Place a carbon-film TEM grid in tweezers under a heat lamp and, while the ultrasonic bath is still operating,

pipette approximately 3 μL of the suspension onto the TEM grid. (Ensure that the pipette tip=s orifice is large enough to accommodate large fibers.) Allow each grid to dry under the heat lamp. A thin carbon coat may be evaporated onto each grid to minimize material loss. Transfer grids to labeled petri dishes or grid box until analysis.

4.6. **Slide Preparation**. *This step will not be necessary for samples that contain more than 1% asbestos as determined by TEM or for samples previously analyzed by the PLM method (Section 4.2 of Item 198.6).* In a few instances, large amphibole fibers at low concentrations may not have been transferred to TEM grids. To avoid overlooking these heavier fibers, sample residue will have to be examined by PLM. A section (4-9 mm^2) of residue should be detached from the polycarbonate filter and placed in a droplet of n_D=1.605 high-dispersion liquid on a clean microscope slide. This residue should be disaggregated by a shearing rubout procedure or similar technique. A coverslip is placed on the preparation and more refractive-index liquid is added at the coverslip edge as necessary.

5. **Sample Analysis**. Each of the prepared grids should be scanned initially at low (~3000) magnification to evaluate the suitability of the preparation. Most grid openings should be covered with intact carbon film and particle distribution should cover 10 to 50% of the grid area. Heavier coverings will tend to make the materials opaque to the electron beam and will make identification and quantitation difficult. Unsuitable preparations must be discarded and additional preparations should be made to compensate for the problems detected on the original preparations, e.g., place a smaller or more dilute subsample on the grid if initial preparations were too heavily loaded. The analyst should also make a presumptive determination of asbestos type(s) present at low magnification before increasing magnification to 10,000 to 20,000 for positive identification. This should be performed on fibers protruding from and representative of large bundles. Finally, the analyst should return to low magnification to estimate the amount of asbestos versus nonasbestos on the entire grid. This procedure must be repeated on the second grid preparation.

5.1. **Identification**. Although this method specifies criteria for positive identification of asbestos, it is not intended as a tutorial in asbestos identification. It is expected that analysts using this method are competent in the identification of asbestos by TEM and can refer to appropriate resource texts for assistance in identification. Some characteristics of asbestos are given in Table I. At least four fibers of asbestos in each sample must be positively identified by each of the criteria required on the analysis sheet:

5.1.1. Morphology.

5.1.2. Electron diffraction data. At least four fibers must be positively identified by electron diffraction.

5.1.2.1. Chrysotile. Minimum requirements for positive identification of chrysotile are:

5.1.2.1.1. Correct (5.3 Å) layer-line (row) spacing.

5.1.2.1.2. Correct orientation of 002, 020, 110, 130 and 200 points or streaks.

5.1.2.2. Amphibole.

5.1.2.2.1. Correct (5.3 Å) layer-line (row) spacing *or*

5.1.2.2.2. Zone-axis measurements that match one of the five asbestos amphiboles.

5.1.3. Elemental composition from energy-dispersive x-ray spectrometer.

5.1.3.1. Chrysotile. (Optional).

5.1.3.2. Amphiboles. Elements critical to the identification of a specific type must be detected at ratios consistent with that type's elemental composition as determined through k-factor calculations.

5.2. **Quantitation**. Matrix losses must be gravimetrically tracked at each preparation step (Section 4.2 through 4.3). Estimates of asbestos percentage versus the percentage of remaining insoluble inorganic matrix material must be made for both of the TEM grid preparations except when final asbestos concentrations will exceed 10%. For these high-concentration ACMs, estimations can be based on a single grid. In making this estimation, the analyst should keep in mind the relative thickness of the different materials on the grid.

The amount of asbestos is then calculated by:

% Asbestos = (PAM/OM)X(AOS)

where:

PAM = Mass of residue after furnace and acid treatments (mg)

OM = Mass of original subsample (mg)

AOS = Mean percentage of asbestos (versus inorganic residue) in both grid preparations

5.3. **Polarized-Light Microscope Analysis**. If TEM analysis yields asbestos concentrations of 1% or less *and* PLM analysis has not been performed in accordance with Section 5.2. of Item 198.6, two coverslip preparations *must* be made according to Section 4.6 and examined by PLM. The entire areas under both coverslips must be examined for traces of asbestos. If any asbestos is detected by PLM, two additional coverslip preparations must be made and all four must be quantitated in accordance with Section 5.2. of ELAP Item 198.6.

5.4. **Analytical Records**. Detailed records must be kept of all phases of the analysis. An analysis sheet that includes all the data required in Section 3.25 must be filled out completely, signed and dated by analyst.

6. **Test Reports**. Reports to clients must include at least the following:

6.1. **Client**. Identify name and address.

6.2. **Sample Identity**. The identification number (Section 8.1.5.1.3) assigned by the laboratory must be clearly cross-referenced to information provided by the client or collector (field identification number, location - Section 8.1.5.1.2)) for each sample.

6.3. **Analytical Results**. The following information must be reported for each sample:

6.3.1. Color of sample.

6.3.2. Presence or absence of asbestos, total percentage of asbestos, type(s) of asbestos present, and percentage of each asbestos type. Asbestos quantities must be reported as one of the following:

6.3.2.1. For samples that have yielded asbestos fibers during preliminary analysis (4.1.3) but have not received full gravimetric reduction, report results as "Asbestos Detected at Unquantified Percentage". Report cannot classify sample as ACM or non-ACM.

6.3.2.2. For samples that contained no asbestos as determined by gravimetric reduction and TEM analysis and had no protruding fibers identified as asbestos during preliminary examination (4.1.3) report: "No asbestos detected".

6.3.2.3. For samples that yielded 1% or less asbestos as determined by gravimetric reduction and TEM analysis *but* had protruding fibers identified as asbestos during preliminary examination (4.1.3) report: "Conflicting results – additional sampling and analysis needed".

6.3.2.4. "Trace **(fill in type(s))** asbestos detected at 1% or less" - for samples that contained 1% or less asbestos.

6.3.2.5. " **(fill in type)** asbestos detected at %" - for each type of asbestos type detected at concentrations greater than 1%.

6.3.2.6. " % Total Asbestos" - sum from all types reported in Section 6.3.2.3. Percentage should be rounded off to two digits.

6.3.3. Type(s) and estimated percentage(s) of other fibrous materials present.

6.3.4. Percentage of nonfibrous matrix material.

6.4. **Homogeneity**. For samples with obvious layers, the summary must include results as specified in Section 6.3 for each layer. Compositing of layers into a single result is no longer allowed, except for joint compounds in certain cases (U.S.E.P.A. 1994b).

6.5. **Disclaimer**. The TEM laboratory must report as verifiable only those operations and analyses performed in-lab. For example, if a filter with residue (Section 4.3 of ELAP Item 198.6) was received from an outside, PLM-only laboratory, the TEM laboratory report should report as verifiable only the percentage of asbestos in that residue.

7. **Precision and Accuracy**.

7.1. **Precision**. Based on limited interlaboratory studies conducted by the New York State Department of Health, quantitative TEM yielded relative standard deviations (RSD) of approximately 45% for samples containing between 15 and 30% chrysotile. Most of the variability was traced to the acid-dissolution/filtration step where RSDs were approximately 25% for 50% carbonate compositions. Agreement during TEM quantitation was better, with about 10% RSDs at 85% chrysotile residues. Chatfield (personal communication) has shown intralaboratory results for a variety of VATs where RSDs were less than 5% for chrysotile concentrations between 10 and 20%.

7.2. **Accuracy**. The accuracy of this method is not completely known at this time. In the same NYSDOH interlaboratory study, gravimetric tracking of the total inorganic residues yielded a mean standard deviation of 6.6% around a mean of 19% for 4 floor tiles. The final analytical step, estimation of asbestos in the inorganic residue, could be complicated by large amounts of non-asbestos that would interfere with detection or by a few large dense particles that might be preferentially rejected during pipetting.

8. Quality Assurance

8.1. **Quality-Assurance Manual**. The laboratory's QA manual can be devoted to asbestos analysis or it can be a larger manual comprising many types of analyses. In either case, the QA manual must include at least the following:

8.1.1. **Quality Assurance Responsibility**. A single individual must be designated as responsible for overseeing quality assurance. This includes updating and controlling distribution of the laboratory's quality-assurance manual, performing at least monthly reviews of analytical quality control and contamination control and resolving any deficiencies.

8.1.2. **Analytical Method**. The laboratory's implementation of the TEM method must be explicitly detailed in this manual. A copy of an externally published method, e.g., this document or the Chatfield (1991) method will not suffice.

8.1.3. **Analytical Quality Control**. The manual must describe a systematic method of submitting quality-control samples including intra-analyst, inter-analyst, standards, proficiency-testing and interlaboratory samples so that analysts are unaware of the sample's true identity.

8.1.4. **Sample Control**. The manual must describe all aspects of bulk sample handling from sample receipt to sample disposal. Criteria for acceptance and rejection of received samples (e.g. broken containers, too-small samples) and for safe handling must be defined. Samples must be retained in secure areas (similar to areas used to store evidentiary material) for at least 60 days after a final report of results is sent to the client. Samples may be returned to the client at the client's request at any time. TEM grids must be stored in a neat and easily retrievable fashion for at least three years after results of analyses have been reported. Procedures for safe disposal of asbestos (in compliance with federal and local regulations) must be detailed and records of such disposals must be kept.

8.1.5. **Recordkeeping**. The laboratory must maintain a recordkeeping system as specified in its QA manual. This must define provisions to ensure the secure storage of records for at least three years. Records, whether they be hardcopy or computer files, must be easily accessible and must include:

8.1.5.1. **Sample Accessioning**. Each sample must pass through an accessioning process that documents:

8.1.5.1.1. **Client**. This should include name, address, phone number and name of contact person.

8.1.5.1.2. **Client Sample Identification**. This should include the identification characteristics provided by the client, e.g., identification number, collection site, etc.

8.1.5.1.3. **Laboratory Sample Identification**. A unique laboratory sample identification number must be assigned to each sample.

8.1.5.1.4. **Date of Receipt**

8.1.5.1.5. **Chain of Custody**

8.1.5.1.6. **Condition of Sample (Accept/Reject)**

8.1.5.1.7. **Type of Sample**. This should place the sample in one of several bulk sample categories, e.g., friable, floor tile, etc.

8.1.5.2. **Analytical Quality Control**. All results of analytical quality control activities must be recorded in an orderly fashion.

8.1.5.3. **Equipment and Supply Records**. Records must be kept for maintenance, calibration, replacement and repair of pertinent equipment and supplies. For major pieces of equipment (microscopes, hoods, muffle furnaces, carbon evaporators, analytical balances) these records must include manufacturer, model and serial numbers, major components, calibration and maintenance/service information and location of manuals.

8.1.5.4. **Contamination Control**. See Section 8.3.

8.1.5.5. **Calibration**. See Section 8.4.

8.1.5.6. **Personnel**. See Section 8.5.

8.1.5.7. **Test Reports**. See Section 6.

8.1.6. **Staff Training Programs**. The Laboratory Director is responsible for continued in-house training of analysts. Analysts must receive formal training in preparation and analysis of samples for asbestos by both TEM and PLM. This can be achieved by sending the analyst to 5-day courses at recognized TEM and/or PLM institutes or by an in-house training course with detailed and extensive curricula equivalent to those at the recognized institutes. The course must include formal training in the theory of

mineral analysis by PLM and TEM and hands-on analysis of all asbestos types, common fiber types and asbestos look-alikes. This formal training must be followed by an in-house apprenticeship during which performance is carefully monitored and documented to show increasing competence to the point where the analyst can work independently within the laboratory's QA framework.

8.2. **Analytical Quality Control**. At least 10% of a laboratory's TEM and PLM analyses must be re-analyzed as part of the laboratory's QC program. Selection of samples for quality-control (intra-analyst, inter-analyst, interlaboratory, or reference) analyses must be semi-random so that the analyst performing the original analysis is not aware that the sample will be reanalyzed. Furthermore, the second analyst must not know the results of the original analysis. These QC data must be routinely assessed to evaluate the precision and accuracy of each analyst and to identify and correct areas of analytical weaknesses. These QC samples must be routinely resubmitted for analytical quality control according to the method detailed in Section 8.1.3. QC reanalysis must include complete gravimetric reduction and re-preparation of TEM grids (and slides, if necessary) from the original sample. All QC results must be documented in a QC notebook or on appropriate analysis sheets. Procedures for resolving analytical discrepancies must be defined and details of resolved discrepancies must be recorded. Discrepancies include classification differences (ACM vs. non-ACM), identification differences (e.g., chrysotile vs. amosite) and substantial quantitation differences, as specified below. Monthly summaries must be compiled for each analyst.

One of QC's primary functions is the timely detection and correction of deficiencies in an analytical system. If QC analysis is not performed concurrent with sample load, QC may be meaningless because analytical conditions at the subsequent time will have changed in some way. Furthermore, if postponed QC uncovers a problem in the analytical system, severe problems may have resulted. Erroneous results may have been reported to a client or additional samples may have been analyzed by the flawed system, resulting in further erroneous data. Thus QC is not an optional activity to be carried out at the convenience of the laboratory or to be postponed when sample loads are heavy. ELAP-certified laboratories **must** perform TEM and PLM QC concurrent with sample load and **must** evaluate these QC results before sending written reports to clients.

8.2.1. **Intra-Analyst QC**. At least 1 out of 50 samples must be reanalyzed by the same analyst. Relative difference (R) values must be calculated for each pair of re-analyses and must be compiled and statistically evaluated for that analyst, comparing his/her QC result to his/her original result for that same sample using:

$R = |(A-B)/((A+B)/2)|$

where

A = First result from the analyst being checked

B = Second result from same analyst for same sample

(Note that these intra-analyst R values are absolute values)

Intra-analyst results will require additional reanalysis, possibly including another analyst, to resolve discrepancies when classification (ACM vs. non-ACM) errors occur, when asbestos identification errors occur, or when R is greater than 1.0.

Record: Sample, date(s) of analyses, analysts' signatures, both results, R value, reason(s) for and resolution(s) of disagreement(s). R control charts must be updated monthly for each analyst monitoring intra-analyst precision. These charts must include all R values from at least the three previous months.

8.2.2. **Inter-Analyst Analysis QC**. At least 1 out of 15 samples must be reanalyzed by another analyst. R values must be calculated for each pair of re-analyses and must be compiled and statistically evaluated for each analyst, comparing his/her result to a QC result for that same sample from another analyst using:

$R = (A-B)/((A+B)/2)$

where

A = Result from the analyst being checked

B = Result from other analyst for same sample

Inter-analyst results will require additional reanalysis, possibly including another analyst, to resolve discrepancies when classification (ACM vs. non-ACM) errors occur, when asbestos identification errors occur, or when R is greater than 1.0 or less than -1.0.

Obviously single-analyst laboratories will not be able to meet this requirement. Instead, they must perform **intra-analyst** analyses on 1 out of every **11** samples.

Record: Sample, date(s) of analyses, analysts' signatures, both results, R value, reason(s) for and resolution(s) of disagreement(s). R-bar control charts must be updated monthly for each analyst monitoring both intra-and inter-analyst precision. These charts must include all R values from at least the three previous months.

8.2.3. **Standard/Reference Analysis**. At least 1 out of 100 samples must be a verified quantitative standard (Section 3.14.2) that has been routinely resubmitted to determine analyst's precision and accuracy. Results should be displayed on x-bar charts to keep track of each analyst's accuracy and precision.

8.2.4. **Interlaboratory QC**. The laboratory must participate in round-robin testing with at least one other ELAP-certified, independent lab. Samples should be sent to these other labs at the rate of 1 sample in 200 routine samples (1 sample in 100 for single-analyst laboratories). These samples must be samples previously analyzed as QC samples. Results of these analyses must be assessed in accordance with QC-outlier criteria detailed in the lab's QA manual. At the very least, the QA manual must address misclassifications (false positives, false negatives) and misidentification of asbestos types.

8.3. **Contamination Control**.

8.3.1. **Prevention**. The laboratory must detail its methods for preventing cross contamination of equipment, supplies and reagents. Much of this will be careful cleaning of work area, equipment and supplies. Intensity and frequency of this effort should be based on experience gained through any contamination detected as described in 8.3.2.

8.3.2. **Monitoring**. The laboratory must have a routine procedure for monitoring contamination of laboratory equipment, supplies and work stations and for resolving contamination problems when discovered. At least one asbestos-free NOB must be prepared and analyzed with every 20 samples analyzed. This non-ACM must go through the full preparation and analysis regimen for the type of analysis being performed. If asbestos is detected, the source of contamination must be traced and the problem resolved to prevent recurrence. Any of the previous samples that may have had results affected by the contamination must be reanalyzed and the client notified of any revisions to reported values. Detailed records of monitoring must be maintained.

8.4. **Calibration**. Written records for calibration of the following equipment and supplies must be kept. All calibrations listed below (unless otherwise noted) must be performed under the same analytical conditions used for routine asbestos analysis and must be recorded in a bound notebook and include date and analyst's signature. Frequencies stated below may be reduced to "before next use" if no samples are analyzed after the last calibration period has expired. Likewise, frequencies should be increased following non-routine maintenance or unacceptable calibration performance.

8.4.1. **Refractive Index Media**. The refractive index medium (oil or solid) used to prepare slides must be calibrated to within 0.004 with certified refractive-index solids or a refractometer. This must be performed when the original container is first opened for use and thereafter at three-month intervals.

Record: Date, nominal refractive index, measured refractive index, temperature.

8.4.2. **Laboratory Thermometer**. The laboratory thermometer must be calibrated to a NIST-traceable standard annually to ±1° C within a temperature range of 20° to 30°C.

Record: Date, nominal temperature from thermometer, actual temperature.

8.4.3. **PLM Alignment**. The PLM must be aligned daily to achieve illumination as close to Köhler illumination as possible and centered through the substage condenser and iris diaphragm. The stage's rotation axis must be centered with the appropriate objectives. Analyzer and polarizer must be rotated to maximum extinction with each other and their privileged directions must be oriented parallel to the lines in the fixed ocular cross hairs (or grid) and aligned at 45° to the accessory port.

Record: Date, check-off for rotation centering, axial illumination, full extinction and crosshair alignment fixed in the polarizer's privileged direction.

8.4.4. **Refractive-Index Colors**. Dispersion-staining or Becke-line colors must be

determined weekly from the permanent 1.680 mount of amosite (Section 3.14.3). The source of any deviations must be located and corrected.

Record: Date, colors or wavelengths perpendicular and parallel to length.

8.4.5. **Analytical Balance**.

8.4.5.1. Analytical balances should be serviced by a qualified service organization annually.

Record: Service organization sticker with date of service.

8.4.5.2. Analytical balances must be checked in two ranges weekly with class S weights. The ranges selected should reflect routine use of the balance and the actual class S weights used should test the scale at mid-point.

Record: Date, target and actual readings in a tabular format.

8.4.6. **Muffle Oven**. Temperatures on external meters (either direct-temperature displays or graduated potentiometers) should be calibrated quarterly (Section 3.7.2). This must be a three-point calibration covering a temperature range of at least 450° to $480^{o}C$. If a thermometer is used for calibration, the thermometer bulb should be immersed in a sand bath.

Record: Date, target temperature, measured temperature in a tabular format.

8.4.7. **HEPA-Ventilated Sample Preparation Enclosure**. Flow rate at enclosure opening shall be measured twice annually to the nearest 5 fpm. Flow rate shall not be less than 75 fpm.

Record: Date, flow rate.

8.4.8. **Transmission Electron Microscope**

8.4.8.1 **Magnification**: Both phosphor viewing screen and micrograph negatives (and/or other hard-copy media) must be calibrated once a month using a calibrated carbon-replica grating at routinely used magnification(s) for accurate measurement of particle dimensions. The calibration system must ensure that 0.5-μm structures (air and water analyses), 5-μm structures (air analysis), and 10-μm structures (water analysis) can be measured with an accuracy of 5% or better at routinely used magnifications.

Record: Nominal TEM magnification(s) vs. a) actual magnification on phosphor screen and b) actual magnification on micrograph negative. Additionally, calibration record must include conversion factors or lengths/diameters of markers on screen used for measuring 0.5 μm, 5 μm (air), and 10 μm (water) structures for ensuring accurate measurements of structures.

8.4.8.2. **TEM Camera Constant**: Both phosphor viewing screen and micrograph negatives (and/or other hard-copy media) must be calibrated using thin-film gold once a week at routinely used camera lengths for accurate measurements of ED

patterns.

Record: Nominal camera length(s) vs. (a) actual camera constant (mmÅ) for phosphor screen and (b) camera constant (mmÅ) for negative. If layer-line spacings are measured on the phosphor screen during routine analysis, the on-screen diameter (mm) of a 5.3 Å ED spacing or an equivalent objective measurement of 5.3 Å spacing must be recorded in the calibration notebook.

Note. Camera-constant calibrations must be performed in the variety of non-eucentric positions expected to be encountered during analysis for those TEMs that cannot always hold specimens at a constant distance from the objective lens, e.g., specimen holders that are not eucentric when tilted. In such configurations where camera constants are not demonstrably reliable to within a ∀5% variation, specimens must be coated with a thin film of gold to provide an internal 0.236 nm calibration standard. This gold film must be thick enough to display the 0.236-nm ring but must be sufficiently thin to minimize masking of weaker diffraction points and absorption of low-energy x rays.

8.4.8.3. **Spot Diameter**: The diameter of the crossover spot used during x-ray analysis must be calibrated every three months and documented by photograph annually.

Record: Diameter (nm) of electron beam (every three months) and photograph with nm or µm scale bar (annually).

8.4.8.4. **Beam Dose**: Low beam dose must be verified every three months. ED patterns from 9 of 10 NIST unit chrysotile fibrils (maximum diameter #0.05 µm) must remain visible for at least 15 seconds.

Record: Electron micrographs of one fibril image and its ED pattern.

8.4.9. **Energy-Dispersive X-ray Analyzer (EDX)**

8.4.9.1. **keV Calibration**: The EDX's multi-channel analyzer calibration must be checked daily by determining keV's at the centers of Cu and Al k_a x-ray peaks generated from specimen(s) in the electron beam. The Cu peak must be adjusted to be within 0.01 keV of 8.04 keV and the Al peak must be adjusted to be within 0.01 keV of 1.48 keV.

Record: Multi-channel analyzer's initial reading (keV) of centers of Cu and Al k_a x-ray peaks and adjustments (if needed).

8.4.9.2. **Detector Resolution**: The resolution of the detector must be verified twice a year to be <175 eV at the full-width half-maximum (FWHM) Mn k_a x-ray peak.

Record: Dated and signed hard copy of keV spectrum showing eV resolution in a notebook.

8.4.9.3. **k Factor**: The k factors to Si for Mg, Ca and Fe must be calculated every

six months using NIST SRM 2063 or 2063a. These factors must be based on peak areas determined by FWHM. The Mg to Fe sensitivity factor must be calculated at the same time and must be 1.5 or less.

Record: Dated and signed hard copy of keV spectrum from 2063, calculations of k and sensitivity factors.

8.4.9.4. **Na Sensitivity**: The low-energy sensitivity of the detector must be verified every three months by producing resolvable Na k_a x-ray peaks from TEM-grid-mounted NIST SRM 1866 crocidolite.

Record: Dated and signed hard copy of 0-10 keV spectrum with resolvable Na k_a x-ray peak in notebook.

8.4.9.5. **Chrysotile Fibril Sensitivity**: The small-structure sensitivity of the detector must be verified every three months by producing resolvable Mg k_a and Si k_a peaks from a single fibril (maximum diameter #0.05 μm) of TEM-grid-mounted NIST SRM 1866, 1876a, or 1876b chrysotile.

Record: Dated and signed hard copy of 0-10 keV spectrum with resolvable Mg k_a and Si k_a peaks with attached electron micrograph of fibril image in notebook.

8.5. **Personnel**. The laboratory must assure that all analysts are competent to perform TEM and/or PLM analysis of asbestos in bulk samples. TEM analysts must be familiar with the theory and interpretation of electron diffraction and understand the relationship between fiber orientation and diffraction orientation in their TEM. PLM analysts must be familiar with the theory of dispersion staining and the measurement of refractive indices by the Becke line technique and be able to apply these. A personnel file must be maintained for every analyst and must include:

8.5.1. **Resume**. Each resume must include formal education, experience and other pertinent information.

8.5.2. **Training**. Both classroom and in-house training must be detailed to demonstrate the analyst's competence in performing independent analysis.

8.5.3. **Job Title**. A job title must be defined that specifies responsibilities and laboratory assignments.

8.5.4. **QC Records**. Details and summaries of results of QC analyses (replicate, duplicate and standard/reference samples) must be updated at least monthly. Precision and accuracy must be determined from the standard/reference samples.

8.5.5. **Deficiency Resolutions**. Details of noted deficiencies and steps taken to resolve these must be included in the personnel folder.

9. **References**

AHERA, 1987. Asbestos Hazard Emergency Response Act, Federal Register, 52(210):41845-41905. Friday, October 30, 1987.

Brzezowski, E.H., 1990. Vinyl asbestos floor tiles: operations and maintenance field test results. Environmental Contractor 2/90:42-104.

Chatfield, E.J., 1991. Determination of asbestos in resilient floor tile. Chatfield Technical Consulting Limited - Standard Operating Procedure SOP 1988-02 Rev.03. Mississauga, Ontario.

Cossette, M., and Delvaux, P. 1979. Technical evaluation of chrysotile asbestos ore bodies, in MAC Short Course in Mineralogical Techniques of Asbestos Determination, R.L. Ledoux (ed.), Mineralogical Association of Canada, Quebec.

Deer, W.A., Howie, R.A., and Zussman, J., 1966. An Introduction to Rock-Forming Minerals, Longman, London.

Marxhausen, T.J., and Shaffer, S.A., 1991. VAT: how safe to maintain? Asbestos Issues 5/91:30-34.

McCrone, W.C., 1980. The Asbestos Particle Atlas. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. 122 pp.

McCrone, W.C., 1988. Asbestos Identification. McCrone Research Institute, Chicago, IL. 199 pp.

MacDonald, H.S., 1991. Asbestos fibers in floor tile: comparison of measurement methods and fiber release from tiles. American Environmental Laboratory 6/91:9-10.

Mullin, C.W., 1988. The problem with vinyl asbestos tile. NAC Journal 6(1):37-39.

NESHAP 1990. National Emission Standards for Hazardous Air Pollutants; Asbestos NESHAP Revision, Final Rule. Federal Register, 55(224):48405-48433. Tuesday, November 20, 1990.

Rajhans, G.S., and Sullivan, J.L. 1981. Asbestos sampling and analysis. Ann Arbor Science, Ann Arbor, MI.

United States Environmental Protection Agency. 1994a. Advisory regarding availability of an improved asbestos bulk sample analysis test method; Supplementary information on bulk sample collection and analysis. Federal Register, 59(146):38970-38971. Monday, August 1, 1994.

United States Environmental Protection Agency. 1994b. Asbestos NESHAP clarification regarding analysis of multi-layered systems. Federal Register, 59(3):542. Wednesday, January 5, 1994.

United States Environmental Protection Agency. 1995. Asbestos NESHAP clarification regarding analysis of multi-layered systems. Federal Register, 60(243):65243. Tuesday, December 19, 1995.

Yada, K. 1969. Study of chrysotile asbestos by a high resolution electron microscope. Acta Crystallographica 23:704-707.

Yada, K. 1971. Study of microstructure of chrysotile asbestos by high-resolution electron

microscopy. Acta Crystallographica A27:659-664.

Bulk Sample Analysis Sheet - Non-Friable Organically Bound

Analyst:____________ **Analysis Date:**__________ **Sample ID:**____________

STEREOBINOCULAR MICROSCOPY:
Color:______ Texture:______ Homogeneity:______ Homogenization:____________
Probable Fibers:__________ Remarks:____________

MATRIX REDUCTION:
ASHING: **g: A:**(Crucible)______ **B:**(Crucible + Subsample)______ **C:**(Crucible + Ashed Subsample)______
ACID DIGESTION: **g: D:**(Filter/Petri)______ **E:**(Filter/Petri + Residue)

CALCULATIONS	g		Percent	
	Calculation	Result	Calculation	Result
Untreated Sample	(**F** = B - A)		(**G** = 100%)	100%
Organic Component	(**H** = (F - (C - A))		(**I** = (H X G) / F)	
Acid-Insoluble Inorganic Component	(**J** = E - D)		(**K** = (J X G) / F)	
Acid-Soluble Inorganic Component	(**L** = (F - (J + H)))		(**M** = (L X G) / F)	

Remarks:____________

TRANSMISSION ELECTRON MICROSCOPE:
IDENTIFICATION: Grid Box/Slot: Grid 1:____/____ Grid 2:____/

Grid	Morphology	SAED Observations	EDX Observations	Remarks	Identity

QUANTITATION:
Estimated Percent Asbestos on: Grid 1: ____ Grid 2: ____ Mean **(N)**: ____
Percent Asbestos in Subsample: (N X K)/G:

POLARIZED-LIGHT MICROSCOPE:
IDENTIFICATION:____________
QUANTITATION:__________ Remarks:__________

FINAL RESULTS:

COMPONENT:	ASBESTOS TYPE	ASBESTOS TYPE	ORGANIC	ACID-SOLUBLE INORGANIC	INSOLUBLE NON-ASBESTOS INORGANIC
PERCENT:					

Verified:__________ **Date:**______ **Remarks:**____________

TABLE I

ASBESTOS TYPE	CHEMICAL COMPOSITION	LATTICE PARAMETERS Δ				JCPDS FILE NUMBER
		a_o	b_o	c_o	a	
Chrysotile (Clino)	$MgSi_2O_5(OH)_4$	5.313 Å	9.120 Å	14.637 Å	93°10'	21-543
Chrysotile (Ortho)	$MgSi_2O_5(OH)_4$	5.340 Å	9.249 Å	14.200 Å	90°	22-1162
"Amosite"	$(Fe_{0.6}Mg_{0.4})_7(OH)_2Si_8O_{22}$	9.534 Å	18.231 Å	5.3235 Å	101.97°	17-726
Crocidolite	$(Na,Ca,K)_{2.2}(Fe^{+2}Mg,Mn,Zn,Cu,Li)_{3.0}(Fe^{+3}Al^{+6}Ti)_{2.0}(Si,Al)_{8.0}O_{22.1}(OH,F)_{1.9}$	9.679 Å	18.048 Å	5.335	103.59°	19-1061
Anthophyllite	$Mg_7Si_8O_{22}(OH)_2$	18.61 Å	18.01 Å	5.24 Å	90°	16-401
Tremolite	$Ca_2Mg_5Si_8O_{22}(OH)_2$	9.879 Å	17.993 Å	5.265 Å	104.151°	20-1310