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1. **Asbestos Analysis by Transmission Electron Microscopy (TEM)**

This supplements the requirements of AHERA (1987) for airborne asbestos analysis and EPA 1993 method for waterborne asbestos analysis. This section does not reduce the QA requirements of AHERA or EPA 1993 method. When airborne asbestos analysis is performed according to the Yamate (1984) method, QA requirements of AHERA must be followed. Items not addressed below must still be followed in accordance with the requirements of the appropriate method.

2. **TEM Specifications**: All TEM's used for asbestos analysis 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).

2.1 **Specimen Holder**: The TEM must have a specimen holder with at minimum a single-tilt capability through at least $\pm 25^{o}$.

2.2 **Phosphor Screen**: 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. **Calibrations**: 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 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 may have to be increased following non-routine maintenance or unacceptable calibration performance. All calibration data must be portrayed on control charts that show trends over time.

3.1. **TEM Calibration**:

3.1.1. **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.

3.1.2. **TEM Camera Constant**: Both phosphor viewing screen and micrograph negatives (and/or other hard-copy media) must be calibrated using thin-film gold or aluminum once a month 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.

3.1.3. **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).

3.1.4. **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.

3.2. **Energy-Dispersive X-ray Analyzer (EDX) Calibration**:

3.2.1. **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_α 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_α x-ray peaks and adjustments (if needed).

3.2.2. **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_α x-ray peak.

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

3.2.3. **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.

3.2.4. **Na Sensitivity**: The low-energy sensitivity of the detector must be verified every three months by producing resolvable Na k_α 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_α x-ray peak in notebook.

3.2.5. **Chrysotile Fibril Sensitivity**: The small-structure sensitivity of the detector must be verified every three months by producing resolvable Mg k_α and Si k_α 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_α and Si k_α peaks with attached electron micrograph of fibril image in notebook.

4. **Analysis**:

4.1. **Grid Types**: Asymmetric finder/relocator/indexed 200-mesh grids must be used to allow subsequent analysts at any laboratory to easily relocate each grid opening previously analyzed. Grid-opening location/number must be recorded on analysis sheets for each grid opening analyzed. Grids must be stored in a neat and easily retrievable fashion for at least three years after results of analyses have been reported.

4.2. **Scanning Method**: When analyzing grid openings, the analyst must use a one-handed stop-and-go movement of one translator when traversing the grid opening. When the grid bar on the other side of the opening is encountered, the specimen is moved down with the other hand so that the returning traverse with the first hand will overlap the previous traverse by about 25%.

4.3. **Analytical Sensitivity**: The combination of volume sampled, filtration area and analysis area for air samples must be such that analytical sensitivity (one counted structure) is less than 0.005 structures (or fibers) per cm^3. Analytical sensitivity for water samples is given in Item 198.2

5. **Auxiliary Equipment**: In order to perform air-sample analysis, a laboratory must have all equipment required in the AHERA **Non**-mandatory method. In order to perform water-sample analysis, a laboratory must have all equipment required in the EPA 1993 method with the exception of the ultraviolet/ozone generator. The ultraviolet/ozone generator is only needed if the samples cannot be filtered within 48 hours. Clarification on specific pieces of equipment are:

5.1 **Low-Temperature Asher Calibration**: The low-temperature asher must be supplied with oxygen and must be calibrated so that 10% of collapsed MCE filters can be removed by controlled etching. This can be accomplished by plotting weight loss of etching of collapsed-filter vs. time. This calibration must be done at least every three months.

Record: Results (total ashing time or calibration curve), recommended settings and time for routine MCE ashing.

5.2 **Carbon Evaporator**: Evaporator must be high-vacuum ($<10^{-4}$ torr) with tilt/rotate capability (EPA 1993 method and AHERA (1987), p. 41880 (III.F.7.c)).

6. **Auxiliary Supplies**: The laboratory must have all supplies required by AHERA for air-sample analysis and by the EPA 1993 water method. The laboratory must also have and use asymmetric finder/relocator/indexed grids (200 mesh) for all routine and QC analyses of air and water samples.

6.1 **Filters**: For filters supplied by the laboratory, the manufacturer's ID and lot number must be recorded on each analysis sheet. At least 2% of laboratory-supplied filters must be prescreened by TEM for appropriate compliance with the method used, e.g., <53 structures/mm^2 for AHERA and <20 fibers/mm^2 for water. Unused portions of filters must be stored in the laboratory for at least 30 days after submitting a written report to the client or the filter may be returned to the client.

6.2 **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.

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

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

7. **Recordkeeping**:

7.1. **Reports to Clients**: Reports to clients must include the electron micrograph number of at least one electron diffraction recorded from each type of asbestos detected in a sample batch.

7.2. **Control Charts**: Control charts must be maintained for calibrations (Section 3) and QC (Section 8) plotting results on a vertical axis versus time on a horizontal axis. These must be updated in a timely fashion before the next calibration or QC is performed.

7.2.1. **Camera Constant**: Camera constant measured at the micrograph level.

7.2.2. **Magnification**: Magnification at the phosphor screen level.

7.2.3. **X-ray Detector Resolution**: Detector resolution at the Mn k_a.

7.2.4. **k Factor**: Ratio of Mg k-factor divided by Fe k-factor.

7.2.5. **Intra-Analyst Precision**: R values for individual analysts.

7.2.6. **Inter-Analyst Precision**: R values for individual analysts versus other analysts.

7.2.7. **Analyst's Accuracy**: Percentage of true positives, false negatives, and false positives as determined by verified asbestos analyses.

8. **Quality Control**: At least 10% of a laboratory's TEM 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. These data must be routinely assessed to evaluate the precision and accuracy of each analyst and to identify and correct areas of analytical weakness. Poisson statistics are not applicable to same-grid-opening recounts (inter-analyst, intra-analyst, interlaboratory QC) but may be applied to repreparations and recounts of different grid openings. QC data must be summarized at least monthly and portrayed through the use of control charts (as outlined in Section 7). Chesson and Chatfield (1989) provide helpful information on TEM QC.

One of QC's primary functions is the timely detection and correction of deficiencies in an analytical system. If QC 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 QC concurrent with sample load and **must** evaluate these QC results before sending written reports to clients.

8.1. **Inter-Analyst**: At least 1 in 25 grid openings or all previously analyzed grid openings in at least 1 in 25 samples must be re-analyzed by another analyst. This reanalysis of the same grid openings will be used to determine both the laboratory's overall precision and to detect bias on the part of different analysts. Relative difference (R) values will be calculated for each pair of re-analyses and will be statistically evaluated for four ranges of average structure counts: <1, 1 to 4.9, 5 to 20, and >20.

For inter-analyst recounts, R values will be calculated for each analyst, comparing his/her results to results from other analysts using:

R = (A-B)/((A+B)/2)
where
A = Result from the analyst being checked
B = Result from other analyst for same sample/grid opening(s)

Inter-analyst counts will require re-analysis in a verified analysis scheme when the appropriate acceptable agreement listed below is exceeded. Appropriate true positives, false negatives, and false positives will then be assigned to each analyst on the basis of their original counts.

Range of Mean of Recount	**Acceptable Agreement**
<5 Structures	±1 Structure
5 to 20 Structures	±2 Structures
> 20 Structures	±3 Structures

Record: Sample, grid(s) and grid opening(s) analyzed, date(s) of analysis, analysts' signatures, both results, R value, reason(s) for and resolution(s) of disagreement(s). A cumulative record of true positive, false positive and false negative results must be kept for each analyst based on re-evaluation of outliers.

8.2 **Intra-Analyst**: At least 1 in 50 grid openings or all previously analyzed grid openings in at least 1 in 50 samples must be re-analyzed by the same analyst. This reanalysis of the same grid openings will be used to determine the analyst's precision. Relative difference (R) values will be calculated for each pair of re-analyses and will be statistically evaluated for four ranges of average structure counts: <1, 1 to 4.9, 5 to 20, and >20.

For intra-analyst recounts, R values will be calculated for that analyst, comparing his/her results to results his/her previous result 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 counts will require re-analysis in a verified analysis scheme when the appropriate acceptable agreement listed below is exceeded. Appropriate true positives, false negatives, and false positives will then be assigned to that analyst.

Range of Mean of Recount	Default Acceptable Agreement
<5 Structures	±1 Structure
5 to 20 Structures	±2 Structures
> 20 Structures	±3 Structures

Record: Sample, grid(s) and grid opening(s) analyzed, date(s) of analyses, analyst's signature, both results, R value, reason(s) for and resolution(s) of disagreement(s). A cumulative record of true positive, false positive and false negative results must be kept for each analyst based on re-evaluation of outliers.

8.3. **Interlaboratory**: The laboratory must participate on a regular basis in interlaboratory quality-control testing with an outside ELAP-approved TEM laboratory. This must involve re-analysis of the same grid openings as in verified analysis. At least two exchanges are required each year, with grid-opening concentrations during each year covering a range that extends from less than 100 to more than 2000 structures per mm^2.

Record: Sample, grid and grid opening identification, date(s) of analysis, in-house analyst's signature, both results, reasons for and resolutions of disagreements.

8.4. **Verified Analysis**: Intra-analyst, inter-analyst and interlaboratory QC as outlined above accounts for 6% cumulative QC. At least an additional 1% QC must be performed as verified analysis as delineated in Steel and Small (1985) and Turner and Steel (1994) on samples with average concentrations between 1000 and 5000 fibers or structures/mm^2 and as verified analysis of NIST reference materials (SRM 1876a, SRM 1876b, RM 8410 or RM 8411) or ELAP proficiency-testing materials. This additional QC must obviously be much higher than 1% for trainees. The remaining 3% of QC must be performed in any of the above categories or in the repreparation and reanalysis of filters or in the analysis of different grid openings from previously analyzed grids. Repreparation data can be used to determine precision associated with filter preparation and fiber distribution while analysis of different grid openings can be used to determine precision associated with fiber distribution on filters.

Record: Results from each analyst, date(s) of analysis, acceptability (within appropriate guidelines), reason(s) for and resolution(s) of disagreements, analysts' signature(s). A cumulative record of true positive, false positive and false negative results must be kept for each analyst.

8.5 **Single-analyst laboratories**: Laboratories that have a single analyst will obviously be unable to perform in-house intra-analyst analyses. Therefore, such laboratories will have to increase their intra-analyst QC to 3% of their sample load, increase their interlaboratory QC to 2% of their sample load, and increase their reference-material QC to 2% of their sample load.

8.6 Each analyst must independently analyze NIST SRM 1876b at least once per year. Results must be within the limits stated on the Certificate.

9. **References**

AHERA, 1987. 40 CFR 763 - "Detailed Procedure for Asbestos Sampling and Analysis - Non-Mandatory" Federal Register, October 30, 1987.

Chatfield, E.J., 1983. Analytical Method for the Determination of Asbestos Fibers in Water. EPA 600/4-84-043.

Chesson, J. and E. Chatfield, 1989. Transmission electron microscopy asbestos laboratories quality assurance guidelines. EPA 560/5-90-002.

Steel, E.B., and J.A. Small, 1985. Accuracy of transmission electron microscopy for the analysis of asbestos in ambient environments. Analytical Chemistry 57(1): 209-213.

Turner, S., and E.B. Steel, 1994. Airborne Asbestos Method: Standard Test Method for Verified Analysis of Asbestos by Transmission Electron Microscopy - Version 2.0. NISTIR 5351.

Yamate, G., 1984. Methodology for the measurement of airborne asbestos by electron microscopy. Draft Report. Washington, D.C.: Office of Research and Development, U.S.E.P.A. Contract No. 68-12-3266.