DATE: | | NVLAP LAB CODE: | |

NIST HANDBOOK 150-13 CHECKLIST

AIRBORNE ASBESTOS ANALYSIS

Instructions to the Assessor: This checklist addresses specific accreditation criteria prescribed in NIST Handbook 150-13, *Airborne Asbestos Analysis* (2006 edition). The numbering of this checklist generally follows the numbering of NIST Handbook 150-13 and contains requirements additional to those in the handbook.

- All items on this checklist shall be addressed.
- Place an “X” beside each item that represents a nonconformity (formerly called "deficiency"); or
  Place a “C” beside each item on which you are making a comment; or
  Place an "OK" beside each item that you observed or verified at the laboratory.
- Record the item number and your nonconformity explanation and/or comments on the comment sheet(s) at the end of this checklist.

3 Accreditation process

3.3 Proficiency testing

The laboratory shall participate in mandatory airborne asbestos proficiency testing when available, which includes (but is not limited to) the following:

_____ 3.3.1 the laboratory shall have written procedures for handling, analysis, and use of NIST proficiency testing materials;

_____ 3.3.2 the laboratory shall keep and use proficiency testing materials as in-house instructional and reference materials, unless otherwise directed;

_____ 3.3.3 all analysts (full- and part-time) shall participate in all proficiency testing rounds with analytical results filed in their personnel folders;

***NOTE**: All analysts need not participate in proficiency testing prior to returning the results to NVLAP, but all analysts shall participate without prior knowledge of the testing results at a later date.*

_____ 3.3.4 each analyst shall separately analyze, record and report test results;

_____ 3.3.5 a single result shall be reported back to NVLAP by the laboratory unless otherwise specified in the testing instructions;

_____ 3.3.6 procedures and calculations (if any) shall be documented as to how a single result was determined;

_____ 3.3.7 test results shall be used for interanalyst comparisons;

_____ 3.3.8 corrective actions shall be taken for interanalyst comparisons;

_____ 3.3.9 plans shall be developed and implemented for resolving problems and shall be documented;

_____ 3.3.10 test results, when applicable, shall be used in determining accuracy and precision for each analyst.

4 Management requirements for accreditation

4.1 Organization

_____ 4.1.1 The laboratory's quality assurance analyses shall represent at least 10 % of the total number of TEM asbestos analyses performed.

NOTE: *The value of 10 % is a minimum value that applies to a laboratory that has: 1) trained analysts, 2) its laboratory calibrations, contamination checks and other quality systems components statistically characterized and in a state-of-control, and 3) a high frequency of analyses. For laboratories not fitting these criteria, the quality assurance analyses must be a higher percentage of the total number of TEM asbestos analyses performed.*

_____ 4.1.2 Quality assurance analyses shall be performed regularly covering all time periods, sample types, instruments, tasks, and personnel. The selection of samples is semi-random and, when possible, the checks on personnel performance are executed without their prior knowledge. A disproportionate number of analyses shall not be performed prior to internal or external audits. Quality assurance analyses shall not be postponed during periods of heavy workloads.

4.1.3 The laboratory shall summarize all of the quality assurance activities each month including:

_____ a) the total number of samples (or grid openings) analyzed;

_____ b) the total number of samples (or grid openings) that have been QC'd;

_____ c) contamination checks, problems, and corrective measures;

_____ d) the total number of interlaboratory and intermicroscope analyses (if applicable);

_____ e) calibrations;

_____ f) identification of any sample custody errors, such as mixing up samples, losing samples, etc.;

_____ g) nonconformity corrections.

4.1.4 The laboratory shall have the latest versions of the following documents available for reference:

_____ a) NIST Handbook 150, *NVLAP Procedures and General Requirements;*

_____ b) NIST Handbook 150-13, *NVLAP Airborne Asbestos Analysis;*

_____ c) the Environmental Protection Agency's, *Interim Transmission Electron Microscopy Analytical Methods—Mandatory and Nonmandatory—and Mandatory Section to Determine Completion of Response Actions*, Appendix A to Subpart E, 40 CFR Part 763, October 30, 1987, or the current U.S. Environmental Protection Agency AEM method for the determination of completion of response actions;

_____ d) *Asbestos-Containing Materials in Schools; Final Rule and Notice*, 40 CFR Part 763, Subpart E;

_____ e) general references on analytical electron microscopy, transmission electron microscopy, asbestos analysis, and crystallography;

_____ f) AEM manufacturer's operation manual;

_____ g) multichannel analyzer manufacturer's operation manual.

4.1.5 The laboratory shall have references available and shall be proficient on the following topics; however, **the exact reference is not required:**

_____ a) **for verified asbestos analysis**, see

- E.B. Steel and J.A. Small, *Accuracy of Transmission Electron Microscopy for the Analysis of Asbestos in Ambient Environments*, Analytical Chemistry, Vol. 57, Issue 1, 1985, pp. 209-213;
- S. Turner and E.B. Steel, *Analysis of Transmission Electron Microscopy Analysis of Asbestos on Filters: Interlaboratory Study,* Analytical Chemistry, Vol. 63, Issue 9, 1991, pp. 868-872;
- S. Turner and E.B. Steel, NISTIR 5351, *Airborne Asbestos Method: Transmission Electron Microscopy – Version 2.0*, 1994;

_____ b) **for spot size measurement**, see

- D.B. Williams, *Practical Analytical Electron Microscopy in Materials Science*, Philips Electronics Instruments, Inc., Mahway, New Jersey, 1984, pp. 34-35 (for TEM or STEM mode);
- David B. Williams and C. Barry Carter, *Transmission Electron Microscopy: A Textbook for Materials Science*, 4 vol. set Plenum Press, New York, 1996 (Paperback);
- D.B. Williams, *Standardized Definitions of X-ray Analysis Performance Criteria in the AEM*, in A.D. Romig Jr. and W.F. Chambers, (ed.), *Microbeam Analysis 1986*, San Francisco Press, San Francisco, 1986, pp. 443-448 (for TEM mode);

- J.I. Goldstein, et al, *Scanning Electron Microscopy and X-ray Microanalysis*, Plenum Press, New York, 1981, p. 48 (for STEM mode);

_____ c) **for k-factor measurement**, see

- D.C. Joy, A.D. Romig, J.I. Goldstein, *Introduction to Analytical Electron Microscopy*, Plenum Press, New York, 1986; or

- D.B. Williams, *Practical Analytical Electron Microscopy in Materials Science*, Philips Electronics Instruments, Inc., Mahway, New Jersey, 1984;

_____ d) **for quality assurance**, see

J.K. Taylor, *Quality Assurance of Chemical Measurements*, Lewis Publishers, Chelsea, Michigan, 1987;

_____ e) **for statistical analysis**, see

M.G. Natrella, *Experimental Statistics*, John Wiley & Sons, New York, 1966;

_____ f) **for control charts**, see

Manual on Presentation of Data and Control Chart Analysis, Seventh Edition, ASTM, Philadelphia, 2002;

_____ g) reference data on the crystallography and chemical composition of minerals that analytically interfere with the regulated asbestos minerals.

4.2 Management system

_____ 4.2.1 If a laboratory is accredited as a sub-facility, it shall be technically dependent on the main facility (i.e., technical management and supervision shall be provided by the main facility).

_____ 4.2.2 Quality assurance activities of the sub-facility shall be directed by the main facility.

4.2.3 The nature, scope, and frequency of on-site quality assurance reviews by the main facility quality manager shall be:

_____ a) clearly defined in the quality manual;

_____ b) appropriate for the nature and scope of work performed by the sub-facility.

_____ 4.2.4 All permanent quality assurance and personnel records shall be retained at the sub-facility and copies provided to the main facility.

_____ 4.2.5 Quality assurance data from each sub-facility shall be compared each month to both the main facility's data and to data from other sub-facilities. Records of such comparisons shall be retained in quality assurance records, along with actions taken to evaluate and resolve differences.

_____ 4.2.6 Analysts at sub-facilities shall participate in NVLAP proficiency testing and records shall be maintained of individual results.

4.3 Document control

(See NIST Handbook 150 Checklist)

4.4 Review of requests, tenders and contracts

(See NIST Handbook 150 Checklist)

4.5 Subcontracting of tests and calibrations

_____ Proficiency testing shall not be contracted out to another laboratory.

NOTE: *A laboratory that subcontracts proficiency testing to another laboratory will be immediately suspended for not participating in the test round and risks revocation of its accreditation.*

4.6 Purchasing services and supplies

(See NIST Handbook 150 Checklist)

4.7 Service to the customer

(See NIST Handbook 150 Checklist)

4.8 Complaints

(See NIST Handbook 150 Checklist)

4.9 Control of nonconforming testing and/or calibration work

(See NIST Handbook 150 Checklist)

4.10 Improvement

(See NIST Handbook 150 Checklist)

4.11 Corrective action

(See NIST Handbook 150 Checklist)

4.12 Preventive action

(See NIST Handbook 150 Checklist)

4.13 Control of records

_____ 4.13.1 All records shall be retained for a minimum of three years and shall be stored in a logical fashion allowing easy retrieval.

_____ 4.13.2 The laboratory shall have documentation, either electronic backup or "paper" hard copy, to ensure survival of original data if computers are used for data retention.

4.13.3 The management system documentation shall contain standardized methods (forms) for recording the following:

_____ a) log-in of samples;

_____ b) criteria for acceptance or rejection;

_____ c) evaluation of quality of prepared grids;

_____ d) AEM sample analysis data.

4.13.4 The laboratory shall have records relating to:

_____ a) sample custody;

_____ b) contamination monitoring.

4.13.5 Records related to contamination shall include results and timing of all checks of the following:

_____ a) filter lot blanks;

_____ b) field blanks;

_____ c) laboratory blanks;

_____ d) all other areas and samples, as needed, to track contamination;

_____ e) summary of contamination problems and resolution;

_____ f) a summary of blank results in control chart or similar format.

4.13.6 All records related to quality assurance testing of staff and laboratory shall be retained including results of:

_____ a) analyses of reference materials;

_____ b) analysis of NIST proficiency testing materials;

_____ c) verified analyses;

_____ d) interlaboratory analyses;

_____ e) intermicroscope analyses (if the laboratory uses more than one AEM for asbestos analysis);

_____ f) repeat preparation and analysis of same filter by same analyst and by different analysts;

_____ g) identification of mineral types;

_____ h) evaluation of filter preparations.

4.13.7 Records related to the AEM analysis of a filter shall include:

_____ a) general information;

_____ operator (analyst must sign and date analysis sheet),

_____ sample identification,

_____ client identification,

_____ date;

_____ b) instrument used (if more than one available);

_____ c) operating parameters of the instrument used including:

_____ magnification,

_____ accelerating voltage,

_____ other, as needed to ensure alignment and calibration compliance;

_____ d) filter- and grid-related information:

_____ filter sampling data sheet as received with sample,

_____ filter type,

_____ area of grid squares analyzed,

_____ number of grids prepared and their grid storage location,

_____ evaluation of prepared grids and grid openings,

_____ orientation of grid in AEM,

_____ grids and grid squares analyzed;

_____ e) original data records include (for AHERA analysis):

_____ structure type (fiber, bundle, cluster, matrix),

_____ the number of fibers that are ≥ 5 micrometers and < 5 micrometers,

_____ the number of fibers that are ≥ 5 micrometers,

_____ classification of structures as chrysotile, amphibole (as grunerite (amosite), riebeckite (crocidolite), anthophyllite, actinolite, or tremolite), or nonasbestos,

_____ at a minimum, one recorded diffraction pattern for each species and one file copy of a spectrum,

_____ at a minimum, measurement results of electron diffraction for each structure (usually the first 4) identified as chrysotile, that caused the concentration of regulated asbestos minerals on the filter to reach or exceed 70 structures/mm^2,

_____ documentation of positive electron diffraction ***or*** EDXA for each chrysotile asbestos structure subsequent to the asbestos structure that caused the concentration on the filter to reach or exceed 70 structures/mm^2, and documentation of positive EDXA or measured zone axis diffraction pattern, for each amphibole structure subsequent to the asbestos structure that caused the concentration of asbestos on the filter to reach or exceed 70 structures/mm^2,

_____ at a minimum, documentation or measurement of results of EDXA and/or measurement of a zone axis electron diffraction pattern for each structure on the non-asbestos class that corresponds to a concentration of over 70 structures/mm^2,

_____ micrograph numbers or appropriate identification for the required one electron diffraction pattern for every five samples that contain asbestos and for any other patterns taken,

_____ interpretation of electron diffraction pattern to verify the identity of asbestos types,

_____ criteria used to classify particles as non-asbestos, that is the property or properties that differentiate it from regulated asbestos minerals;

NOTE: *For structures whose qualitative chemical composition is distinct from regulated asbestos minerals, e.g., gypsum, only documentation of the qualitative chemical composition is necessary. For structures that have similar qualitative composition, semiquantitative measurement of composition by EDXA and/or measurement of the distinguishing features of the diffraction pattern is required.*

Measurement results shall include sufficient quantitative data (e.g., x-ray intensities or diffraction maxima d-spacing) to identify regulated asbestos minerals positively, as defined by laboratory identification criteria. Documentation of positive diffraction or EDXA means that the analyst records (e.g., checks off) that these properties visually and/or qualitatively match the lab's identification criteria.

_____ f) information related to report to client:

_____ concentration of asbestos in structures/mm^2 on filter and structures/cm^3 in sampled air,

_____ number of asbestos structures counted,

_____ types of asbestos.

_____ area analyzed,

_____ volume of air sampled.

_____ 4.13.8 A cumulative record of results from precision and accuracy testing shall be maintained and summarized at least monthly.

4.14 Internal audits

(See NIST Handbook 150 Checklist)

4.15 Management reviews

(See NIST Handbook 150 Checklist)

5 Technical requirements for accreditation

5.2 Personnel

_____ 5.2.1 Staff members shall be aware of both the extent and the limitation of their area of responsibility.

_____ 5.2.2 The laboratory shall have a written description of its training program that includes training with standards and blind testing, and NVLAP documentation, to determine competency and criteria for successful completion.

_____ 5.2.3 Analysts and technical supervisors shall participate in an appropriate form of continuing education, such as formal coursework, in-house education, and scientific or technical meetings, and have access to journals that describe advances in the field of electron microscopy and/or asbestos analysis.

_____ 5.2.4 The technical supervisor(s) shall be qualified to conduct AEM studies, apply AEM to crystalline materials and shall be proficient in the field of asbestos analysis including procedures for sample handling, preparation, analysis, storage, disposal, and contamination monitoring.

5.2.5 AEM analysts shall be trained and be proficient in:

_____ a) AEM use, calibration, alignment, electron micrography (or functional equivalent);

_____ b) EDXA, x-ray collection and interpretation including the formation of EDX patterns, recognition of artifacts and abnormal features in spectra resulting from detector problems, contamination or detector-sample geometry;

_____ c) selected area electron diffraction measurement and interpretation including the formation of SAED patterns, determination of d-spacings, and Miller indices of individual diffraction spots and the corresponding zone axis;

d) asbestos counting methods including:

_____ counting rules for simple and complex structures;

_____ grid and grid square selection (nonadjacent, random);

_____ x-y stage translation and parallel traverses;

_____ stage positioning and repositioning;

e) asbestos identification including:

_____ morphology criteria;

_____ crystallographic criteria through selected area electron diffraction analysis;

_____ chemical composition criteria through EDXA;

_____ f) differentiation between regulated asbestos minerals and other minerals that resemble the regulated asbestos minerals;

_____ g) determination of the concentration of fibers on a filter sample;

_____ h) verified asbestos analysis;

_____ i) recognition of acceptable and unacceptable sample preparations;

_____ j) recognition of sample and instrumental artifacts;

_____ k) AHERA clearance protocols.

5.2.6 The accuracy of asbestos identification and counting of each AEM analyst shall be evaluated by:

_____ a) analyses of reference materials prepared in-house and purchased;

_____ b) analyses of NIST proficiency testing samples; and

_____ c) verified analyses.

NOTE: *Additional quality assurance information may be gained by determining the precision of AEM analysts by repeat analysis of grid squares.*

5.2.7 Verified asbestos analyses shall be performed routinely by the laboratory with sufficient frequency and on sufficient types of samples to determine each operator's initial and continuing performance that the following conditions are satisfied:

_____ a) samples having approximately 6–40 structures/grid opening shall be used to achieve statistically significant information on new analysts;

_____ b) after initial training, a variety of asbestos loadings, including routine AHERA samples, shall be used to validate analysts' results. Samples shall include loadings seen in typical AHERA samples up to 6–40 structures/grid opening. At least 20 % of verified analyses shall be performed on samples with 6-40 structures/grid opening loadings and at least 5 grid openings with 6-40 structures/grid opening shall be counted annually;

_____ c) filter blanks, unless known to be contaminated, shall not be used for verified counting;

_____ d) during training, all counts used in reports shall be verified until verified status is attained;

_____ e) after verified status is attained, the frequency of verified analysis shall be at least 1 per 100 grid opening analyses.

NOTE: *Labs may find it advantageous to use as many as four operators on one analysis to characterize initial operator performance, if an analyst with verified status is not available. An analyst that obtains verified status shall have an average accuracy ≤ 80 % of true positives, ≤ 20% false negatives, and ≤ 10 % false positives on both standard and field samples.*

_____ 5.2.8 The laboratory shall have a person responsible for tracking and storing samples (a laboratory sample coordinator).

_____ 5.2.9 The laboratory shall be organized so that staff members are not subject to undue pressure or inducement that might influence their judgment or results of their work. The laboratory is able to demonstrate that the sample work required for each analysis is consistent with accurate and precise analytical measurements.

5.3 Accommodation and environmental conditions

5.3.1 The following facilities shall be available:

_____ a) clean room or clean areas for sample preparation and handling separate from bulk asbestos;

_____ b) electron microscopy facility;

_____ c) room or area for filter and grid storage separate from bulk asbestos.

5.3.2 The following shall be available in the clean room or clean area:

_____ a) class 100 (or cleaner) HEPA-filtered air under positive pressure, which shall be maintained except for maintenance;

_____ b) exhaust hood for safe use of filter dissolution reagents.

5.3.3 Safe working conditions shall be maintained, including:

_____ a) safe handling of asbestos;

_____ b) safe handling and storage of filter-dissolving reagents such as chloroform, dimethyl formamide, acetone, acetic acid, etc.

5.3.4 Management system documentation shall contain:

_____ a) procedures for the prevention, monitoring, and control of contamination of filters and grids;

_____ b) procedure (or flow chart) for the systematic checking for possible sources of contamination if contamination is detected. This includes checking all areas, instrumentation and materials used in the preparation and analysis of air filter samples.

5.3.5 To minimize the possibility of contamination of samples, the following shall be performed:

_____ a) all personnel shall be instructed in contamination prevention;

_____ b) personnel, instrumentation and materials used for the preparation of bulk materials that potentially contain asbestos shall be kept separate from areas used for air filter preparation, handling or analysis;

_____ c) all reagents shall be checked for asbestos contamination prior to use in sample preparation, or purchased from previously tested sources in previously tested grades.

NOTE: *Personnel who have worked with bulk samples should not subsequently be allowed to work with air filter samples on the same day. It is acceptable, however, for personnel to work on bulk samples after having worked with air samples.*

NOTE: *The next four items concern the use of blank materials for contamination monitoring. Definitions of filter lot, sealed, field and laboratory blanks are given in 1.5 of NIST Handbook 150-13. Other blanks should be used as needed to determine and correct sources of contamination, including blanks for AEM specimen holders, evaporators, Jaffe wick, low temperature asher, laboratory air samples, etc.*

_____ 5.3.6 A laboratory blank filter shall be present in the clean room or clean area during sample preparation and after cleaning or servicing of the clean room or clean area. The laboratory blanks shall be obtained from a filter lot that has been shown not to be contaminated.

5.3.7 The maximum allowed contamination levels for filter lot, sealed, field and laboratory blanks shall be:

_____ a) for polycarbonate filters — a cumulative average level of 18 structures per mm^2;

_____ b) for mixed cellulose ester — a cumulative average of < 5; single < 15.

_____ c) a single preparation level of 53 structures per mm^2.

5.3.8 Preparation of nominally blank filters shall be done:

_____ a) on a minimum of one laboratory blank per sample set or 10 % of samples (whichever is greater);

_____ b) on a laboratory blank after cleaning or servicing the clean room or clean area;

_____ c) on the field and sealed blanks with each series of samples (if these blanks are not identified and known to the laboratory, all filter samples are prepared with the series).

NOTE: *The laboratory shall properly record and archive prepared grids (even if not analyzed).*

5.3.9 Analysis of nominally blank filters shall be done:

_____ a) polycarbonate filters — on a minimum of one laboratory blank per 25 filter analyses;

_____ b) mixed cellulose ester — on a minimum of one laboratory blank per 100 filter analyses;

_____ c) on the laboratory blank when the average count for the full set of filters exceeds 70 structures/mm^2;

_____ d) on the field and sealed blank when the full indoor/outdoor analysis is performed.

NOTE: *If the Z-test is performed by the laboratory, then the field and sealed blanks must be known to the laboratory. The laboratory is responsible for the analysis of the filter lot blanks only when contracted to analyze them by the sampling organization.*

The laboratory blank analyses may be counted towards the required 10 % quality assurance analyses. The field and sealed blank analyses; however, may not be counted towards this requirement.

_____ 5.3.10 When contamination above acceptable levels is found, analyses for AHERA clearance shall be discontinued until the cause is found and corrected or until data shows the contamination problem no longer exists.

5.4 Test and calibration methods and method validation

(See NIST Handbook 150 Checklist)

5.5 Equipment

5.5.1 The following sample preparation equipment or equivalent shall be available in the clean room or area:

_____ a) condensation washer and/or Jaffe wick with the appropriate reagents and supplies;

_____ b) filter preparation materials (e.g., scalpel, microscope slides, tweezers, etc.);

_____ c) indexed 200-mesh TEM grids—also referred to as finder grids (only grids with unique identifiable grid openings may be used; grids with only a symmetrical central marking do ***not*** qualify as finder grids);

_____ d) other materials as needed.

5.5.2 The laboratory shall have a low temperature plasma asher which:

_____ a) is supplied with oxygen;

_____ b) allows for control of speed of evaluation and venting to minimize disturbance of particles on filter surface;

_____ c) is not used for bulk samples (asbestos or other).

5.5.3 The laboratory shall have:

_____ a) a carbon evaporator which attains a vacuum of 13 Pa (10^{-4} torr) or lower and has controlled venting to atmospheric pressure;

_____ b) spectrochemically pure carbon rods;

_____ c) a carbon rod sharpener;

_____ d) gold or aluminum wire for evaporation (or have sputter coater with gold target).

5.5.4 The laboratory shall have an electron microscope, which has the following under routine asbestos analysis conditions:

_____ a) capability of operation at a voltage between 80 keV-120 keV;

_____ b) capability of producing an electron diffraction pattern of a single fibril of all regulated asbestos minerals;

_____ c) capability of displaying and resolving hollow tube of chrysotile;

_____ d) capability of precise fiber length (at 0.5 µm) and diffraction pattern measurement, regardless of image (fiber or pattern) orientation (often fulfilled through use of a fluorescent screen with calibrated gradations in the form of circles or at least two perpendicular lines);

_____ e) mechanical stage with linear, reproducible movements along two perpendicular directions;

_____ f) capability of producing a spot at crossover that is ± 250 nm during EDXA analysis;

_____ g) an imaging system for recording brightfield images and electron diffraction patterns on electron micrographs or on other suitable media.

NOTE: *It is strongly recommended that the laboratory possess a holder capable of obtaining zone axis diffraction patterns (either a double-tilt or rotation-tilt holder).*

5.5.5 The laboratory shall be able to record and produce hard copies of images (on electron micrographs or other media) to document:

_____ a) visibility of chrysotile hollow tubes and beam damage;

_____ b) visibility and measurement of electron diffraction patterns, in particular chrysotile (002), (004), (110), (020), (130), and (200) reflections;

_____ c) complex arrangement of fibers;

_____ d) a range of magnifications from 1000 x to 100 000 x in brightfield imaging mode;

_____ e) a range of diffraction camera lengths that enable accurate diffraction pattern measurement (approximately 20 cm to 80 cm).

_____ 5.5.6 An EDXA system shall be interfaced to all electron microscopes used for asbestos analysis.

5.5.7 The EDXA system (detector and multichannel analyzer), under routine analysis conditions, shall meet the following specifications:

_____ a) 175 eV or better resolution at Mn Kα peak;

_____ b) proven statistically significant detection of Na Kα peak in standard crocidolite or equivalent;

_____ c) capable of obtaining statistically significant Mg and Si peaks from a single fibril of chrysotile;

_____ d) consistent relative sensitivity factors over large areas of the specimen grid (see 5.6.1.12).

NOTE: *(1) A low background holder may be necessary to meet these requirements, (2) for item 5.5.7b, the Na K-lines and Cu L-lines (potentially from the Cu TEM grid) have significant overlap and care must be taken to show that Na is measured above the Cu L-line background.*

5.5.8 The multichannel analyzer shall have the following:

_____ a) software capable of obtaining background corrected peak intensities or integrals for Na, Mg, Al, Si, Ca, Fe and other elements as needed;

_____ b) capability of accumulation and display of an x-ray spectrum (minimum 0.7 keV-10 keV);

_____ c) capability of making a hard copy of an x-ray spectrum.

5.6 Measurement traceability

5.6.1 General

NOTE: *Control charts shall be constructed to show calibration values vs. time, the magnitude of their variation, and the allowable limits of variation. The magnitude of variation specified for many calibrations in this program is defined as 2 s (s is the estimated standard deviation of a set of measurements). Initially, many (15-30) calibrations should be performed in a few months' time to establish a baseline for variation in the measurements. If the variation is within specified limits and the accuracy is acceptable, the frequency of the calibration can be reduced. In general, the majority of calibrations should have been done within 3 months prior to analyses performed for clients.*

All calibrations shall be performed with the instrument, stage, sample, x-ray detector and other parameters at routine asbestos analysis conditions (e.g., tilt, apertures, location,

specimen height, accelerating voltage, etc.) and with the microscope aligned. Tilting the viewing screen and specimen grid during fiber measurement, or the viewing screen during diffraction measurement is not recommended. Laboratories using tilts must demonstrate the required measurement accuracy and precision for all fiber and diffraction maxima orientations.

_____ 5.6.1.1 The laboratory shall have specific procedures in its management system documentation for the development and use of control charts, including the algorithms for calculating warning and control limits.

_____ 5.6.1.2 The laboratory shall use control charts to summarize all calibration data.

5.6.1.3 The magnification of the electron microscope shall be calibrated:

_____ a) using an optical diffraction grating replica (the variation in the calibration measurements, i.e., two times the standard error (2 *s*) is < 5 % of the mean calibration value);

_____ b) for magnifications commonly used for asbestos analysis and for any other magnification used for measurement (e.g., the magnification used to measure grid square size);

_____ c) on all measurements systems applied in the laboratory for asbestos analysis such as the phosphor viewing screen, film, monitor and/or image analysis system.

5.6.1.4 The accuracy and precision of measurements at 0.5 μm shall be determined by:

_____ a) calibration of the measuring system(s) (on screen, film, monitor, and/or image analysis system) at 0.5 μm;

_____ b) repeat analysis by the same and different analysts of asbestos fibers approximately 0.5 μm in length. (This data may be derived in part from verified analysis data for fibers close to 0.5 micrometers in length.)

5.6.1.5 The diffraction camera constant shall be calibrated:

_____ a) using an evaporated gold or aluminum film (the variation in the calibration measurements (2 *s*) is < 5 % of the mean calibration value);

_____ b) for the camera lengths commonly used for asbestos analysis;

_____ c) under the conditions used for asbestos analysis;

_____ d) on all measurement systems including the TEM screen, film, monitor, image analysis system and/or any other system as applied in the laboratory for asbestos analysis.

NOTE: *A minimum of three measurements at 45° angles is required on the innermost ring. These measurements will allow detection of deviations of a ring from a circle. Measurements of at least two of the outer rings must be made to monitor for radial distortions and to ensure that an error in measurement of the inner ring did not occur. If significant distortion is found, more measurements are needed for better characterization.*

_____ 5.6.1.6 The beam dose shall be calibrated so that beam damage to chrysotile is minimized—specifically so that an electron diffraction pattern from a single fibril $\geq$ 1 μm in length from a NIST SRM chrysotile sample is stable in the electron beam for at least 15 seconds.

_____ 5.6.1.7 The laboratory shall have recorded the setting of the electron microscope (condenser aperture, spot size, etc.) that allows for the stability of chrysotile as specified in item 5.6.1.6 above. This setting shall be used as the standard operation procedure for routine analyses of possible chrysotile structures. For microscopes that cannot scan at electron fluxes which limit the damage to chrysotile, a procedure how to limit damage (e.g., immediately defocus the condenser) is required.

5.6.1.8 The spot size of the electron beam used for well resolved x-ray microanalysis shall be determined and the:

_____ a) the average spot size for a properly stigmated beam is $\leq$ 250 nm;

_____ b) the variation in diameter measurements (2 *s*) is < 25 % of the mean value.

5.6.1.9 The EDXA system shall be shown through calibration data to have:

_____ a) a resolution (full-width, half-maximum) for Mn Kα that is < 175 eV;

_____ b) a value for the sum of the resolution and the variation (2 *s*) that is < 180 eV for $\leq$ 5 measurements taken on a given day.

_____ 5.6.1.10 The x-ray energy vs. channel number for the EDXA system shall be calibrated to within 20 eV for at least two peaks between 0.7 keV and 10 keV. One peak shall be from the low end (0.7 keV to 2 keV) and the other peak from the high end (7 keV to 10 keV) of this range. The calibration of the x-ray energy shall be checked prior to each analysis of samples and recalibrated if out of the specified range.

5.6.1.11 The relative sensitivity (k-factors) factors relative to Si for elements found in asbestos (Na, Mg, Al, Si, Ca, Fe) shall be determined so that:

_____ a) the k-factors are determined to a precision (2 *s*) within 10 % relative to the mean value obtained for Mg, Al, Si, Fe, and within 20 % relative to the mean value obtained for Na;

_____ b) the k-factor relative to Si for Na is between 1.0 and 4.0, for Mg and Fe is between 1.0 and 2.0, and for Al and Ca is between 1.0 and 1.75;

_____ c) the k-factor for Mg relative to Fe on SRM 2063(a) or other standard traceable to NIST is 1.5 or less.

NOTE: *SRM 2063 or SRM 2063a can be used for the determination of k-factors for Mg, Si, Ca and Fe. The laboratory must obtain its own chemically characterized materials for determining the Na and Al k-factors. Examples include albite for Na k-factor determination and biotite or albite for Al k-factor determination. Na k-factors are sensitive to electron beam does (current and time). It is suggested that small particles (≤ 0.1 µm in size) be used for Na k-factor determination to minimize the effect of Na migration.*

_____ 5.6.1.12 The portions of a grid in a specimen holder for which abnormal x-ray spectra are generated under routine asbestos analysis conditions shall be determined and these areas avoided in asbestos analysis.

NOTE: *X-rays can be absorbed due to the relative position of the area of interest, the grid bars, specimen holder and x-ray detector and give an abnormal spectra (for an example of an abnormal spectra see S. Turned, E.B. Steel, S.S. Doorn, and S.B. Burris, "Proficiency Tests for the NIST Airborne Asbestos Program – 1991," NISTIR 5432). The laboratory should use a standard material (SRM 2063 is recommended) to map out the spectra obtained over the grid area and to thereby determine the regions that should be avoided in routine analysis.*

_____ 5.6.1.13 The low temperature asher shall be calibrated by determining a calibration curve for the weight vs. ashing time of collapsed mixed-cellulose-ester (mce) filters.

NOTE: *The AHERA method specifies that a mixed-cellulose-ester filter is to be ashed by 10 %. However, if ashing by this amount generates a texture in the replica that affects structure counting, it is permissible to etch by less than this amount.*

5.6.1.14 The determination of the quality of sample preparations shall be calibrated or the laboratory shall have the following documentation available:

_____ a) images and samples showing good preparations and examples of the types of problems that occur in poor preparations (readily available to analysts);

_____ b) a record of repeat evaluations of images and samples by the same and different analysts. (This data may be derived in part from sample preparation evaluations done in the course of verified analysis.)

_____ 5.6.1.15 The magnification of the grid opening measurements system shall be calibrated using an appropriate standard. The variation in the calibration measurements (2 *s*) is < 5 % of the mean calibration value.

_____ 5.6.1.16 Trained AEM analysts shall have an average accuracy ≥ 80 % of true positives, ≤ 20 % false negatives, and ≤ 10 % false positives (these data are reported on a structures per grid square basis).

_____ 5.6.1.17 The laboratory and AEM analysts shall obtain mean analytical results on SRM 1876b so that trimmed mean values fall within 80 % of the lower limit and 110 % of the upper limit of the 95 % confidence limits as published on the certificate (these limits are derived from the allowable false positives and false negatives given in the previous item). The SRM shall be analyzed a minimum of once a year by each AEM analyst.

_____ 5.6.1.18 The laboratory shall have documentation demonstrating that AEM analysts correctly classify at least 90 % of both bundles and single fibrils of asbestos structures ≥ 1 µm in length in known standard materials traceable to NIST (such as the bulk asbestos SRM 1866).

_____ 5.6.1.19 Interlaboratory analyses shall be performed to detect laboratory bias. The frequency of interlaboratory verified analyses shall correspond to a minimum of 1 of 200 grid square analyses for clients.

_____ 5.6.1.20 If more than one AEM is used for asbestos analysis, intermicroscope analyses shall be performed to detect instrument bias.

_____ 5.6.1.21 The sampling precision shall be determined by repeat preparation and analysis of the same filter by the same and different analysts.

_____ 5.6.1.22 Required calibrations shall be performed correctly and on a frequent enough basis to ensure accurate results.

5.6.2 Calibration

_____ 5.6.2.1 The laboratory shall use Environmental Protection Agency, “Interim Transmission Electron Microscopy Analytical Methods—Mandatory and Nonmandatory—and Mandatory Section to Determine Completion of Response Actions,” Appendix A to Subpart E, 40 CFR Part 763, October 30, 1987, and any NIST or U.S. Environmental Protection Agency clarifications, modifications, or updates to the TEM method for the analysis of asbestos.

_____ 5.6.2.2 Management system documentation shall detail the AEM method as it is applied in the laboratory. (A simple copy of the AHERA method is not sufficient). If departures are made from the method, the laboratory shall have written procedures detailing how the analyses are conducted.

5.6.2.3 The laboratory shall have written procedures for:

_____ a) preparation of mixed-cellulose-ester filters, including techniques for collapsing, etching, carbon coating and dissolution of filters;

_____ b) preparation of polycarbonate filters, including techniques for carbon coating and dissolution of filters;

_____ c) determination of the number of grid squares and grid area to be analyzed per sample.

5.6.2.4 Laboratory personnel shall:

_____ a) prepare and store at least three grids per filter;

_____ b) analyze approximately half of the predetermined sample area to be analyzed on one grid and the remaining half on a second grid preparation.

5.6.2.5 The laboratory shall have written procedures for the evaluation of the quality of prepared grids. The criteria for acceptance include:

_____ a) the percentage of grid openings covered by the replica section (coherent or noncoherent) is greater than approximately 50 %;

b) the percentage of grid openings covered by the replica section that:

_____ are intact is greater than approximately 50 %;

_____ have undissolved filter that is less than approximately 50 %;

_____ have overlapping or folded replica that is less than approximately 50 %.

_____ c) at least 20 grid squares have no overlapping or folded replica, < 5 % holes and < 5 % opaque area due to incomplete filter dissolution. “Opaque area” means that the sample preparation artifact is sufficiently opaque to the electron beam that recognition and analysis of fibers will be difficult or impossible.

_____ 5.6.2.6 The laboratory shall have written procedures for the determination of the area of grid squares.

NOTE: *The AHERA method requires that either 1) the area of the grid square analyzed be determined, or 2) the average area of grid squares in a grid lot of 1000 be determined by measurement of 20 grid squares on each of 20 grids. If premeasured grids are purchased, the laboratory should confirm the measurements as a quality assurance procedure. Initially, many grid squares should be remeasured. If the values are in agreement with those given with the grids, then the number of remeasurements can be reduced to approximately 5 % of those required by the AHERA method. Premeasured grids must have a report of analysis, which gives the mean grid opening area, the number of grids and openings measured, the standard deviation of the opening area, and the method of analysis.*

5.6.2.7 The laboratory shall have written procedures for operation of the AEM for asbestos analysis including:

_____ a) method for alignment of the electron microscope so that the electron beam travels down the optic center of the column (this includes alignment of the electron gun, apertures, and tilt as described in the manufacturer’s and laboratory’s operating manual);

b) standard operating conditions of the AEM

_____ i) voltage (between 80 keV-120 keV)

_____ ii) microscope magnification (15 000 x –20 000 x for analysis).

_____ 5.6.2.8 There shall be documentation to show that the quality of alignment of the electron microscope is checked daily or prior to each use for analyses and calibrations. The alignment is checked by, at minimum, changing the magnification, spot size, and image focus and by checking the stigmation of the electron beam. The AEM analyst aligns the electron microscope if the instrument does not meet the laboratory's alignment criteria as stated in the quality manual. (Note: Alternatively, the AEM can be aligned daily or prior to each use).

5.6.2.9 The laboratory shall have written procedures for examining a grid square and for counting and analyzing particles (a detailed description is necessary—a copy of the EPA method is not sufficient) including:

_____ a) method for recording grid orientation in the microscope;

_____ b) particle loading acceptance criteria (> 10 % by area particulate loading or uneven particle loading is rejected, see 5.6.2.5);

_____ c) unique grid and grid square labeling system (indexed grids);

_____ d) grid square traversing method, including the use of orthogonal scans;

NOTE: *The intent is to completely cover the grid square without having structures missed or counted twice. To do this, parallel, overlapping traverses are made across a grid square. Care is taken to move only one translator during a traverse. If an asbestos structure is encountered and the other translator is moved for analysis, then the stage is returned to the original traverse position before continuation of the traverse.*

_____ e) recording rules;

_____ f) structure counting rules;

_____ g) determination of whether a sample set passes or fails AHERA clearance if required by the client.

5.6.2.10 The laboratory shall have management system documentation which contains criteria for:

_____ a) identification of electron diffraction patterns of regulated asbestos minerals and of nonasbestos minerals, including those that closely resemble regulated asbestos minerals;

_____ b) identification of EDXA spectra of regulated asbestos minerals and of nonasbestos patterns, including those that closely resemble regulated asbestos minerals;

_____ c) differentiating asbestos minerals from at least the following phases; the pyroxenes, hornblende, wollastonite, halloysite, palygorskite, sepiolite, antigorite, lizardite, talc, and vermiculite. The minimum criteria for differentiation must be presented.

_____ 5.6.2.11 AEM analysts shall record sufficient information for each analysis so that a verified analysis can subsequently be performed.

NOTE: *Information sufficient for performing a verified analysis includes the orientation of the grid at the analysis magnification, a sketch (or image) for each structure and the size of each structure (the recording of the location of the structure is also of use). Recording this information will allow for random quality assurance checks of any analysis and removes the bias that can occur when verification is done with the analysts' foreknowledge. The laboratory may want to refer to E.S. Windsor, S. Turner and E.B. Steel, NISTIR 5358, in which a recording form suitable for verification is described.*

NOTE: See Appendix A of this checklist.

_____ 5.6.2.12 AEM analysts shall record a selected area electron diffraction pattern of one asbestos structure on from every five samples that contain asbestos. The identification of diffraction patterns shall be verified by a qualified individual. It shall be shown that AEM analysts are correct 80 % of the time in identification of recorded diffraction patterns.

_____ 5.6.2.13 AEM analysts shall record an EDS spectrum from one asbestos structure of each type of amphibole asbestos from every set of samples that contain asbestos. The identification of EDS spectra shall be verified by a qualified individual. It is shown that the AEM analysis is correct 80 % of the time in identification of EDS spectra.

_____ 5.6.2.14 The laboratory shall have written procedures for acquiring, recording, indexing, and interpreting SAED patterns, including Miller indices of diffraction spots and, for amphiboles, zone axis.

_____ 5.6.2.15 The laboratory shall have written procedures for verifying report calculations.

5.6.3 Reference standards

5.6.3.1 The following standards and any associated certificates shall be on hand:

_____ a) materials with a certified value for the loading of asbestos on filters, such as NIST SRM 1876b, if available;

_____ b) materials that are characterized as asbestos for training and analyst evaluation such as NIST SRMs 1866 and 1867 or NIST-traceable standard, if available;

_____ c) calibration material(s) for the x-ray system such as SRM 2063 or NIST-traceable standard, if available;

_____ d) standard optical grating replica or calibrated polystyrene spheres for magnification calibration;

_____ e) gold or aluminum film material for electron diffraction calibration.

NOTE: *(1) The laboratory must use SRM 1876b for calibration and not SRMs 1876 or 1876a. SRM 1876 and 1876a were certified using sets of counting rules that are no longer in use. (2) SRMs 1866 and 1867 contain bulk asbestos and, therefore, precautions need to be taken against contaminating the filter preparation area and AEM with these specimens. (3) The laboratory has the primary responsibility for developing or obtaining a set of standards useful for checking the identification, analysis and concentration of asbestos on filters. For example, internal standards can be drawn from samples received by the laboratory or developed by the laboratory through water filtration of asbestos mixtures or by other methods. The samples then must be well-characterized by the laboratory for use as standards. NVLAP proficiency testing samples do not qualify as NIST-traceable standards.*

5.7 Sampling

(See NIST Handbook 150 Checklist)

5.8 Handling of test and calibration items

5.8.1 The log-in system shall include documentation of:

_____ a) the date of receipt;

_____ b) identity of the client;

_____ c) unique identification for sample;

_____ d) air volume pulled through sample;

_____ e) filter pore size;

_____ f) condition of the samples; and

_____ g) acceptance or rejection of the samples.

_____ 5.8.2 The laboratory shall have written criteria for acceptance or rejection of filter cassettes.

NOTE: *Examples of rejection criteria include: insufficient sampling documentation, bulk samples included with air filter samples, filter cassettes open, filters overloaded with particulate, uneven particle loading, sampling parameters not meeting AHERA sampling criteria, filters not uniquely identified, filters of incorrect pore size, tampering with cassettes evident, sample that laboratory is not capable of preparing properly, etc.*

5.8.3 The laboratory shall have a documented chain-of-custody system by which the following is recorded:

_____ a) location of sample;

_____ b) a listing of personnel that have handled or worked with the sample;

_____ c) a listing of what has been done to the sample.

5.8.4 The laboratory shall:

_____ a) store the unused portions of filters in their cassettes for at least 30 days;

_____ b) store all prepared grids (even if not analyzed) for at least three years;

_____ c) store the filters and grids in a logical fashion so that specified samples can be retrieved within one working day.

5.9 Assuring the quality of test and calibration results

(See NIST Handbook 150 Checklist)

5.10 Reporting the results

5.10.1 Test reports shall include the following information for each sample set:

_____ a) area of filter analyzed;

_____ b) volume of air sampled (with reference to sampling data sheet);

_____ c) analytical sensitivity used for the analysis;

_____ d) number of total asbestos structures and number of structures by asbestos type (chrysotile, grunerite, riebeckite, anthophyllite, tremolite, or actinolite);

_____ e) concentration in asbestos structures/mm^2 of filter and asbestos structures/cm^3 of air for total asbestos structures, and with data broken down by size (≥ 5 µm and ≥ 0.5 µm to < 5 µm), and by asbestos type;

_____ f) statement of analytical uncertainty, including 95 % confidence limits on the reported concentration and laboratory and analyst accuracy and precision;

_____ g) micrograph number of any recorded diffraction patterns;

_____ h) copy of AEM analysis data record with analyst's signature or initials;

_____ i) descriptions of any departures from the test method.

5.10.2 The following additional information shall be supplied if asbestos abatement clearance is determined to be necessary:

_____ a) calculation formulas;

_____ b) all calculation variables and constants;

_____ c) all calculation results.

APPENDIX A

Verified Asbestos Analysis

_____ **A.1 Verified analysis**

Verified asbestos analysis is currently the best way to compare results among analysts and to check for the accuracy of an analyst on an unknown sample (see Steel, E. B. and J. A. Small, in clause 1.4). It consists of multiple operators independently analyzing a grid square, comparing the results, and requires that beam currents be low enough that at least two consecutive analysts can observe electron diffraction patterns from the same fiber.

Laboratories may find it advantageous to use several operators in a verified analysis of a grid square to characterize initial operator performance if an analyst with verified status is not available (to achieve verified status an AEM analyst shall attain an average accuracy of ≥ 80 % true positives, ≤ 20 % false negatives, and ≤ 10 % false positives on verified analyses). The number of analysts who can analyze a grid square is limited by beam damage to the particles (four operators is typically the maximum number of analysts that can be used). Multiple analysts from different laboratories can be used if the initial orientation of the grid and grid opening is recorded and made known to subsequent laboratories.

One of the most common causes of false negatives in an AEM analysis of asbestos occurs when the operator fails to find or observe a fiber or structure. This is commonly due to the operator missing a whole or partial traverse of the grid square. A source of both false negatives and false positives is the individual operator's interpretation of asbestos structure counting rules as it is applied to complex structures. These types of errors can be uncovered and corrected by the use of verified analyses. As analysts within a laboratory count structures in a more uniform manner, the imprecision (due to the analysts) within the laboratory will decrease.

Verified asbestos analyses shall be performed by the laboratory on a routine basis and on sufficient types of samples to determine each operator's initial and continuing performance. Samples having approximately 6-40 structures/grid opening shall be used to achieve statistically significant information on new analysts. During training, all counts used in reports shall be verified until verified status is attained. After initial training, a variety of asbestos loadings, including routine AHERA samples, shall be used to validate analysts' results. At least 20 % of verified analyses shall be performed on samples with 6-40 structures/grid opening loadings and at least 5 grid openings with 6-40 structures/grid opening shall be verified annually. Filter blanks, unless known to be contaminated, shall be rarely used for verified counting. After verified status is attained, the frequency of verified analysis shall be at least 1 per 100 grid opening analyses.

_____ **A.2 NISTIR 5351**

It is suggested that TEM laboratories follow NISTIR 5351, *Airborne Asbestos Method: Standard Test Method for Verified Analysis of Asbestos by Transmission Electron Microscopy - Version 2.0.* (Available from NVLAP.)

APPENDIX B

TEM Grid Square Overloading Criterion

B.1 Introduction

When grids are prepared for TEM asbestos analysis, some of the grid squares may be observed to be heavily loaded with particulate matter. Overloaded squares are not to be analyzed, so there must be a criterion to define which squares are overloaded and must be rejected. In both the NVLAP TEM NIST Handbook 150-13 Checklist and the Asbestos Hazard Emergency Response Act (AHERA), the overload criterion is stated as a percentage of the grid square that is covered with particulate matter, but there is a discrepancy between the criteria stated in the NVLAP 150-13 Checklist and the AHERA. Item 5.6.2.9 b) in the Handbook 150-13 Checklist required grid square with > 10 % by area particulate loading to be rejected for analysis, while the AHERA puts the rejection criterion at > 25 % by area particulate loading.

This has led to some disagreement among TEM laboratories, their clients, and NVLAP assessors regarding the overload criterion; is the NVLAP requirement 10 % or 25 %?

B.2 NVLAP requirement

The NVLAP requirement is 10 % coverage, just as written in the NIST 150-13 Checklist, Item 5.6.2.9. Item 5.6.2.9 requires laboratories to have written procedures for examining a grid square and for counting and analyzing particles. Part b) under 5.6.2.9 states "particle loading acceptance criterion (> 10 % by area particulate loading or uneven particle loading is rejected)." Thus, for NVLAP TEM laboratories, the 10 % criterion is to be written into their procedures, and the laboratories will be assessed to the 10 % requirement.

The NIST 150-13 Checklist requirements were written after careful study of AHERA and examination of numerous prepared grids. In the case of the criterion for acceptance/rejection of loaded grid squares, the Technical Experts at NIST decided 25 % or greater overloading coverage would allow asbestos structures to be obscured, so a lower coverage of 10 % was adopted as the NVLAP requirement to prevent the underestimation of asbestos structure concentrations during routine analysis of airborne asbestos samples.

COMMENTS AND NONCONFORMITIES

Instructions to the Assessor: Use this sheet to document comments and nonconformities. For each, identify the appropriate item number from the checklist. Identify comments with a "C" and nonconformities with an "X." If additional space is needed, make copies of this page (or use additional blank sheets).

Item No.	Comments and/or Nonconformities