**NATIONAL ENVIRONMENTAL LABORATORY ACCREDITATION CONFERENCE (NELAC)**

**ON-SITE LABORATORY ASSESSMENT**

**ASBESTOS MICROSCOPY CHECKLIST (16 PAGES TOTAL)**

LABORATORY: _____

Physical Address: _____

_____

Mailing Address: _____
(if different from above)

_____

Telephone Number: _____ Facsimile Number: _____

E-mail address: _____

INSPECTED BY: (Name) (Affiliation)

_____ _____

_____ _____

_____ _____

INSPECTION DATES: _____

LABORATORY TECHNICAL DIRECTORS AND MANAGEMENT:

(Name) (Title)

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

GENERAL INSTRUCTIONS: Before each item is a blank line and a NELAC Standard citation in **Bold Numerals**.

Place a check mark ( __---- ) in the blank if the laboratory meets the NELAC Standard referenced.

Place an X-mark ( X ) in the blank if the Standard is not met and the laboratory must devise an acceptable Plan of Correction and estimated completion date. **The NELAC Standard reference must be cited in in the on-site assessment report.**

Mark **"N/A"** in the blank if the NELAC Standard is not applicable to this laboratory, either because of the nature of its business mission, because of the analytical tests it performs, or because of the situation never ever happening.

**Notes:**

The use of **EPA Methods 100.1 & 100.2** for SDWA are **mandated at 40 CFR Part 141.23(k)**

If the laboratory appears to meet a particular NELAC Standard but does not have the documentation to back up its claim, use the following:

_____ **5.0** Does the laboratory have **all items** identified in NELAC Chapter 5 Quality Systems **available** for on-site inspection or data audit

**ASBESTOS LABORATORY TOUR**

____ **D** Does the laboratory have **procedures** for developing **acceptance/rejection criteria** for each test method

____ **5.5.3.2** Does the laboratory meet & document adherence to **laboratory facility requirements** specified by the test method
**Note: 40 CFR Part 763, App. A to Subpart E** requires sample preparation area for air samples in a **separate room** from where bulk Asbestos samples are prepared

____ **D.6.4(b)** Does the laboratory use the results of **proficiency test sample analysis** to evaluate its ability to **produce accurate data**

____ **D.6.9** Has the laboratory established & adhered to written procedures to **minimize** the possibility of **cross-contamination** between samples

COMMENTS:

## ASBESTOS TEST METHODS

____ **5.5.4.1.2(a)** Does the laboratory have an **in-house methods manual** for each accredited **analyte** or **method**
**Note:** This manual may consist of copies of published or referenced test methods

____ **5.5.4.1.2(b)** Does the laboratory **clearly indicate** in its methods manual **any modifications** made to the referenced test method and **describe any changes or clarifications** where the referenced test method is ambiguous or provides insufficient detail

Does each test method in the in-house methods manual include or reference, where applicable:

____ **5.5.4.1.2(b)(1)** **Identification** of the test method
____ **5.5.4.1.2(b)(2)** Applicable **matrix or matrices**
____ **5.5.4.1.2(b)(3)** **Method Detection Limit**
____ **5.5.4.1.2(b)(4)** **Scope & application**, including components to be analyzed
____ **5.5.4.1.2(b)(5)** **Summary** of the test method
____ **5.5.4.1.2(b)(6)** **Definitions**
____ **5.5.4.1.2(b)(7)** **Interferences**
____ **5.5.4.1.2(b)(8)** **Safety**
____ **5.5.4.1.2(b)(9)** **Equipment & supplies**
____ **5.5.4.1.2(b)(10)** **Reagents & standards**
____ **5.5.4.1.2(b)(11)** Sample **collection, preservation, shipment, & storage**
____ **5.5.4.1.2(b)(12)** **Quality control**
____ **5.5.4.1.2(b)(13)** **Calibration & standardization**
____ **5.5.4.1.2(b)(14)** **Procedure**
____ **5.5.4.1.2(b)(15)** **Calculations**
____ **5.5.4.1.2(b)(16)** Method **performance**
____ **5.5.4.1.2(b)(17)** Pollution **prevention**
____ **5.5.4.1.2(b)(18)** **Data assessment & acceptance criteria** for quality control measures
____ **5.5.4.1.2(b)(19)** **Corrective actions** for out-of-control data
____ **5.5.4.1.2(b)(20)** Contingencies for **handling out-of-control or unacceptable data**
____ **5.5.4.1.2(b)(21)** **Waste management**
____ **5.5.4.1.2(b)(22)** **References**
____ **5.5.4.1.2(b)(23)** **Tables, diagrams, flowcharts, validation data**

____ **5.5.9.2(d)** Does the laboratory's **Asbestos data** indicate that the **quality control protocols** in the test methods manual **are being followed**

____ **D** Does the laboratory **assess & evaluate** all **quality control measures** on an **on-going basis**

____ **D** Does the laboratory **use** quality control **acceptance criteria** to determine the **validity of the data**

____ **D** Does the laboratory ensure that the **essential standards** outlined in Appendix D are incorporated into the method manuals and/or Quality Manual

COMMENTS:

ASBESTOS TEST METHOD ASSESSED: _____

____ **5.5.4.2.2(a) C.1 D.6.4(a)** Has the laboratory performed a **satisfactory demonstration of method capability** prior to the acceptance & institution of this test method

____ **C.1** Does the laboratory **document** in its Quality Manual **other adequate approaches** to **Demonstration of Capability** if the procedure in Appendix C is **not required** by the mandated test method or regulation and if the laboratory **elects not to perform** this procedure

____ **5.5.4.2.2(d) C.2** Does the laboratory use the **NELAC-specified certification statement** to document the **completion of each Demonstration of Capability** (initial & continuing)

____ **C.2** Are copies of these certification statements retained in the **personnel records** of each **employee performing the test method**

____ **5.5.2.6(c)(3)** Does each Analyst have **documentation** of **continued proficiency** by at least **one of the following once per year**:

- Acceptable performance of a **blind sample** (single blind to the analyst)
- Another **demonstration of capability or initial measurement system evaluation**
- Successful performance of a blind performance sample on a **similar test method** using the **same technology** (acceptance limits must be determined prior to analysis)
- At least **4 consecutive** laboratory **control samples** with **acceptable levels** of precision & accuracy (acceptance limits must be determined prior to analysis)
- Analysis of **authentic samples** that have been analyzed by **another trained analyst** with **statistically identical results**

____ **5.5.4.2.2(d)** Does the laboratory **retain all associated supporting data** necessary to **reproduce the analytical results** summarized in the appropriate certification statement

____ **5.5.4.2.2(e) C.1** Does the laboratory **complete a demonstration of capability each time** there is a **change** in **instrument type, personnel, or test method**

____ **5.5.4.2.2(f)** Does the laboratory **fully document** the achievement of **demonstration of capability requirements** for each **specialized work cell**

**Note:** A work cell is defined as a group of analysts with specifically defined tasks that together perform the test method

____ **5.5.4.2.2(g)** Does the laboratory demonstrate & document acceptable performance through **acceptable continuing performance checks** (e.g laboratory control samples) **each time** that **membership** in a work cell **changes**

____ **5.5.4.2.2(g)** Do the **new members** of the work cell **work with experienced analysts** in the specialty area

____ **5.5.4.2.2(g)** Does the laboratory **repeat a Demonstration of Capability** with the new work cell if the **first 4 continuing performance checks** following the change in personnel **produce a failure** in any sample batch acceptance criteria

____ **5.5.4.2.2(g)** Does the laboratory **repeat a Demonstration of Capability** if the entire **work cell is changed or replaced**

____ **5.5.4.2.2(h)** Is the **performance of the work cell** as a group **linked to the training records** of the **individual members** of the work cell

## TRANSMISSION ELECTRON MICROSCOPY (TEM)

**Negative Controls**

____ **D.6.1.1.1(a)** Are **at least 4 glass sample bottles & at least 1 polyethylene sample container** per set of 24 such sample containers tested for **background asbestos levels** prior to sample collection of **waters & wastewaters**

____ **D.6.1.1.1(a)** Is the **bottle blank level** of Asbestos less than or equal to 0.01 MFL (million fibers per liter) for fibers greater than 10 um in length

____ **D.6.1.1.1(b)** Is a **process blank sample** consisting of asbestos-fiber-free water run before the first field sample
**Note:** The quantity of water filtered must be at least 10 mL for a 25-mm diameter filter & 50 mL for a 47-mm diameter filter

____ **D.6.1.1.2(a)** Is a **blank filter** prepared & analyzed with each set of **20 or fewer air samples analyzed**
**Note:** The blank filter must be left uncovered during preparation of the sample set, and a wedge from that blank filter must be prepared & analyzed alongside wedges from the sample filters

____ **D.6.1.1.2(b)** Is the **maximum Asbestos contamination** on a single blank filter no more than **53 structures per square millimeter**

____ **D.6.1.1.2(b)** Is the **maximum average Asbestos contamination** for all blank filters no more than **18 structures per square millimeter**

____ **D.6.1.1.3(a)** Are **contamination checks** using asbestos-free material (e.g., a glass fiber blank) performed at a frequency of at least **one per every 20 bulk Asbestos samples analyzed**

____ **D.6.1.1.3(a)** Is an **investigation done** to detect & remove the source of Asbestos contamination when the contamination check **detects Asbestos** at a concentration **exceeding 0.1%**

____ **D.6.1.1.3(b)** Does the laboratory **maintain a list of non-Asbestos fibers** that can be confused with Asbestos
**Note:** The list must include crystallographic and/or chemical properties that disqualify each fiber being identified as Asbestos

**Test Variability / Reproducibility**

____ **D.6.2.1** Are quality assurance analyses **performed regularly** covering all time periods, instruments, & personnel

____ **D.6.2.1** Is the selection of samples **random** for quality assurance analyses

____ **D.6.2.1** When possible, are checks on personnel performance executed **without their prior knowledge**

____ **D.6.2.1** Is a **disproportionate number** of analyses **not performed** prior to internal & external audits

____ **D.6.2.1.1** **D.6.2.1.2** Are all **water, wastewater, & air analyses** performed on **relocater grids** so that other laboratories can easily repeat analyses on the same grid openings

____ **D.6.2.1.1** Are quality assurance analyses **not postponed** during periods of heavy workloads of water & wastewater sample analyses

____ **D.6.2.1.1** Does the total number of QA samples & blanks for water & wastewater analyses **exceed 10%** of the total sample workload

____ **D.6.2.1.2** If more than one TEM is used for Air Asbestos analyses, does the laboratory perform **intermicroscope analyses** to detect instrument bias

____ **D.6.2.1.2** Does the laboratory & each TEM analyst for **air samples** analyze **NIST SRM 1876b annually** to obtain trimmed mean analytical results within **80% of the lower limit & 110% of the upper limit** of the 95% confidence limits as published on the certificate

____ **D.6.2.1.2** Does the laboratory have documentation demonstrating that TEM analysts for Air samples **correctly classify** at least 90% of both bundles & single fibrils of Asbestos structures greater than or equal to 1 um in length in **known standard reference materials** (e.g., NIST bulk Asbestos SRM 1866)

____ **D.6.2.1.2** Are **interlaboratory analyses** performed to detect laboratory bias

____ **D.6.2.1.2** Does the frequency of interlaboratory verified analyses correspond to a **minimum of 1 per 200 grid square analyses** for clients

____ **D.6.2.1.1(a)** Is a **second, independent analysis** performed on the same grids but **on different grid openings**
**D.6.2.1.2(a)** than used in the original analysis of water, wastewater, & air samples, at a frequency of **at least one per 100 samples**

____ **D.6.2.1.1(a)** Are the results of this Replicate analysis within **1.5 times the Poisson standard deviation**

____ **D.6.2.1.1(b)** Is a **second aliquot** of water or wastewater sample **filtered through a second filter**, prepared, & analyzed in the same manner as the original preparation of that sample, at a frequency of **at least one per 100 samples**

____ **D.6.2.1.2(b)** Is a **second wedge** from an air sample filter prepared & analyzed in the same manner as the original preparation of that sample, at a frequency of **at least one per 100 samples**

____ **D.6.2.1.1(b)** Are the results of this Duplicate analysis within **2.0 times the Poisson standard deviation**
**D.6.2.1.2(b)**

____ **D.6.2.1.1(c)** Is a **second, independent analysis** performed on the **same grids & grid openings** used in the
**D.6.2.1.2(c)** original analysis of water, wastewater, & air samples, at a frequency of **at least one per 20 samples**
**Note:** The 2 sets of results must be compared according to Turner & Steel (NISTIR 5351)

____ **D.6.2.1.1(c)** Do the **qualified Asbestos analysts** maintain an average of at least **80%true positives,** less than
**D.6.2.1.2(c)** **20% false negatives**, & less than **10% false positives**

____ **D.6.2.1.3** Is **at least 30% of the laboratory's bulk sample QC analyses** performed on samples containing **1-10% Asbestos**

____ **D.6.2.1.3(a)** Are **at least one out of 50** bulk Asbestos samples **reanalyzed by the same analyst**
**Note:** For single-analyst laboratories, this frequency must be at least one every 10 bulk samples

____ **D.6.2.1.3(b)** Are **at least one out of 15** bulk Asbestos samples **reanalyzed by another analyst**

____ **D.6.2.1.3(b)** Are **additional reanalyses performed**, possibly including another analyst, to **resolve discrepancies** when classification errors occur, when asbestos identification errors occur, or when inter-analyst precision is found to be unacceptable

____ **D.6.2.1.3(c)** Does the laboratory **participate in round robin testing** with at least one other laboratory, with bulk Asbestos samples sent to this laboratory **at least 4 times per year**

____ **D.6.2.1.3(c)** Are the bulk Asbestos round robin samples **previously analyzed as QC samples**, with the results of round robin analyses **assessed in accordance with QC requirements**

**Note:** As a minimum, the QC requirements must address misclassifications (false positives, false negatives) & misidentification of asbestos types

____ **D.6.3.1.1(a)** Are filter preparations made **from all 6 Asbestos types** from NIST SRM 1866 & SRM 1867, with
**D.6.3.1.2(a)** **concentrations between 1-20 structures** (greater than 10 um in length) per 0.01 square millimeters

____ **D.6.3.1.1(a)** Is one such filter preparation **analyzed independently** at a frequency of **one per 100 samples**
**D.6.3.1.2(a)** (waters, wastewaters, & air) analyzed, with results evaluated as verified asbestos analysis in accordance with Turner & Steel (NISTIR 5351)

____ **D.6.3.1.1(b)** Is **NIST SRM 1876b** analyzed **annually by each analyst**, with results evaluated in accordance with
**D.6.3.1.2(b)** limits published for that SRM

**Note:** This SRM may not be appropriate for aqueous Asbestos analysis, but this SRM is the only recognized TEM counting standard

____ **D.6.3.1.3** Are all analysts able to **correctly identify the 6 related Asbestos types** (chrysotile, amosite, crocidolite, anthophyllite, actinolite, & tremolite)

**Asbestos Calibration**

____ **D.6.5.1.1** Are all calibrations performed **under the same analytical conditions** used for routine Asbestos analysis

**Note:** Except as noted below, these standards refer to TEM calibrations for all waters, wastewaters, air, & bulk samples

____ **D.6.5.1.1** Are all calibrations **recorded in a notebook** and include **date & analyst's signature**

____ **D.6.5.1.1(a)** Is **magnification calibration** done at the fluorescent screen, with the calibration specimen at the eucentric position, at the magnification used for fiber counting
(generally 10000-20000x for water & wastewater, 15000-20000x for air & bulk samples)

____ **D.6.5.1.1(a)** Is a **logbook maintained** with the **dates** of magnification calibration **recorded**

____ **D.6.5.1.1(a)** Are the magnification calibrations **performed monthly** to establish the stability of magnification

____ **D.6.5.1.1(a)** For water & wastewater samples, is the magnification calibration data **displayed on control charts** to show trends over time

____ **D.6.5.1.1(b)** Is the **camera length** of the TEM in the **Selected Area Electron Diffraction (SAED) mode** calibrated before SAED patterns of unknown samples are observed

____ **D.6.5.1.1(b)** Is the diffraction specimen **in the eucentric position** for this calibration

____ **D.6.5.1.1(b)** Do these Camera Constant calibrations **allow accurate (<10% variation) measurement** of layer-line spacings **on the medium used for routine measurement** (i.e., the phosphor screen or camera film), plus **accurate (<5% variation) measurement** of zone axis SAED patterns **on permanent media** (e.g., film)

____ **D.6.5.1.1(b)** Are the calibrations **performed monthly** to establish the stability of the camera constant

____ **D.6.5.1.1(b)** Where non-asbestiform minerals may be expected (e.g., winchite, richterite, industrial talc, vermiculite, etc.), is an **internal camera constant standard** (e.g., gold) deposited & measured **on each sample** to facilitate accurate indexing of zone axis SAED patterns
**Note:** In such cases, layer-line analysis alone shall not be used

____ **D.6.5.1.1(b)** Is Camera Constant calibration data **displayed on control charts** that show trends over time

____ **D.6.5.1.1(c)** Is the diameter of the **smallest beam spot at crossover** less than **250 nm**, as **calibrated quarterly**

____ **D.6.5.1.1(c)** Is this Spot Size calibration data **displayed on control charts** that show trends over time

____ **D.6.5.1.1(d)** Is the **beam dose** calibrated so that **beam damage to chrysotile is minimized**, such that an electron diffraction pattern from a single fibril greater than 1 um in length from a NIST SRM chrysotile sample is stable in the electron beam dose for **at least 15 seconds**

____ **D.6.5.1.1(e)(1)** Is the **x-ray energy vs. channel number** for the EDXA system calibrated to **within 20 eV** for at least 2 peaks **between 0.7 keV & 10 keV**

____ **D.6.5.1.1(e)(1)** Is one peak for the EDXA calibration **from the low end** (0.7-2.0 keV) & the other peak **from the high end** (7-10 kEV) of this range

____ **D.6.5.1.1(e)(1)** Is the calibration of the x-ray energy **checked prior to each analysis** of samples & **recalibrated** if out of the specified range

____ **D.6.5.1.1(e)(2)** Is the ability of the EDXA system to **resolve the Na k-alpha line from the Cu L line** confirmed **quarterly** by obtaining a spectrum from the NIST SRM 1866 crocidolite sample on a copper grid

____ **D.6.5.1.1(e)(3)** Are the **k-factors** for elements commonly found in Asbestos (Na, Mg, Al, Si, Ca, & Fe) relative Si **calibrated semiannually**, or anytime the detector geometry is altered

____ **D.6.5.1.1(e)(3)** Is **NIST SRM 2063a** used for evaluating the k-factors for Mg, Si, Ca, & Fe
**Note:** k-factors for Na & Al may be obtained from albite, kaersutite, or NIST SRM 99a

____ **D.6.5.1.1(e)(3)** Are the k-factors determined to a **precision (2-sigma) within 10%** relative to the mean value obtained for Mg, Al, Si, Ca, & Fe and **within 20%** relative to the mean obtained for Na

____ **D.6.5.1.1(e)(3)** Is the k-factor for **Na relative to Si** between 1.0-4.0

____ **D.6.5.1.1(e)(3)** Are the k-factors for **Mg & Fe relative to Si** between 1.0-2.0

____ **D.6.5.1.1(e)(3)** Are the k-factors for **Al & Ca relative to Si** between 1.0-1.75

____ **D.6.5.1.1(e)(3)** Is the k-factor for **Mg relative to Fe** less than or equal to 1.5

____ **D.6.5.1.1(e)(3)** Is the EDXA calibration data **displayed on control charts** that show trends over time

____ **D.6.5.1.1(e)(4)** Is the EDXA **detector resolution checked quarterly** to ensure a full-width half-maximum resolution **less than 175 eV** at the Mn k-alpha x-ray emission (5.90 keV)

____ **D.6.5.1.1(e)(4)** Is the EDXA detector resolution checks **displayed on control charts** that show trends over time

____ **D.6.5.1.1(e)(5)** Does the laboratory determine portions of a TEM grid for which **abnormal x-ray spectra** are generated under routine Asbestos analysis conditions & **avoid these areas** in Asbestos analysis

____ **D.6.5.1.1(e)(6)** Is the sensitivity of the EDXA detector for collecting x-rays from small volumes **documented quarterly** by collecting **resolvable Mg & Si peaks** from a unit fibril of NIST SRM 1866 chrysotile

____ **D.6.5.1.1(f)** Is the **low-temperature asher calibrated quarterly** by determining the calibration curve for the weight vs. ashing time of collapsed mixed-cellulose-ester (MCE) filters

____ **D.6.5.1.1(f)** Is this asher calibration data **displayed on control charts** that show trends over time

____ **D.6.5.1.1(g)** Is the **magnification of the grid opening measurement system** calibrated **quarterly** using an appropriate standard at a frequency of **20 openings per 20 grids per lot** of 1000 TEM grids, or **one opening per sample**

____ **D.6.5.1.1(g)** Is the **variation** in the Grid Opening calibration measurements (2-sigma) **less than 5%** of the mean calibration value

**Analytical Sensitivity**

____ **D.6.6.1.1** Is an **analytical sensitivity of 200000 fibers per liter** achieved for each **water & wastewater** sample analyzed

**Note:** Sensitivity is defined is defined as the concentration represented by finding one Asbestos structure in the total area of filter examined

____ **D.6.6.1.2** Is an **analytical sensitivity of 0.005 structures per square centimeter** achieved for each **air** sample analyzed

**Data Reduction**

____ **D.6.7.1.1(a)** **D.6.7.1.2(a)** **D.6.7.1.3(a)** Is the concentration of Asbestos in a given sample **calculated in accordance with the method** utilized (refer to NELAC Section 5.5.4.7.2 for additional data reduction requirements)

____ **D.6.7.1.1(b)** **D.6.7.1.2(b)** Does the laboratory calculate & report the **upper & lower 95% confidence limits** on the mean concentration of Asbestos fibers found in the sample

**Quality of Standards & Reagents**

____ **D.6.8.1(a)** Does the laboratory quality control program **establish & maintain** provisions for **Asbestos standards**

____ **D.6.8.1(a)(1)** Are the **Asbestos reference standards** obtained from NIST, EPA, or suppliers who participate in supplying NIST standards or NIST-traceable Asbestos

**Note:** Any reference standard purchased outside the United States must be traceable to that country's national standards laboratory; commercial suppliers of reference standards must conform to ANSI N42.22 to assure the quality of their products

____ **D.6.8.1(a)(2)** Are these reference standards accompanied with a **certificate of calibration** whose content is as described in ANSI N42.22-1995, Section 8, Certificates

____ **D.6.8.1(b)** Are all reagents used in Asbestos analysis **analytical reagent grade** or better

____ **D.6.8.1(c)** Does the laboratory have mineral fibers or data from mineral fibers that will **allow differentiating Asbestos** from at least the **following look-alikes**: fibrous talc, sepiolite, wollastonite, attapulgite (palygorskite), halloysite, vermiculite scrolls, antigorite, lizardite, pyroxenes, hornblende, richterite, winchite, or any other asbestiform minerals suspected as being present in the sample

## PHASE CONTRAST MICROSCOPY

**Negative Controls:**

____ **D.6.1.2** Are at least **2 field blanks** (or 10% of total samples, whichever is greater) submitted for analysis with **each set** of samples

**Note:** Field blanks are handled in the same manner as the associated samples, except that air is not drawn through the blank sample; the blank cassette is opened for about 30 seconds at the same time that other cassettes are opened just prior to analysis

____ **D.6.1.2** Is the identity of the blank filters **unknown to the counters** until all counts have been completed

____ **D.6.1.2** Does the laboratory report **possible contamination** of the samples if a field blank yields greater than **7 fibers per graticule fields**

**Note:** Results from field blank samples are used in the calculation to determine final airborne fiber concentration

**Test Variability / Reproducibility**

____ **D.6.2.2(a)** Does the laboratory implement an **interlaboratory quality control program** that includes participation of **at least 2 other independent laboratories**

____ **D.6.2.2(a)** Does the laboratory participate in **round robin testing every 6 months** with those other laboratories, by submitting slides typical of its own workload for use in this program

____ **D.6.2.2(a)** Is the round robin designed & results analyzed with **appropriate statistical methodology**, with the **results posted in each laboratory** to keep the microscopists informed

____ **D.6.2.2(b)** Does each analyst select & **count a prepared slide** from a reference slide library on **each day** on which air counts are performed

____ **D.6.2.2(b)** Are the Asbestos **reference slides** prepared using **well-behaved samples** taken from the laboratory workload, with the **fiber densities** covering the **entire range** routinely analyzed by the laboratory

____ **D.6.2.2(b)** Are the reference slides **counted by all analysts** to establish an original standard deviation & corresponding limits of acceptability

____ **D.6.2.2(b)** Are the results of the daily reference sample analysis **compared to the statistically derived acceptance limits** using a control chart or database

____ **D.6.2.2(b)** Is the inter-analyst precision **posted in each laboratory** to keep the microscopists informed

____ **D.6.3.2(a)** Are **blind recounts** by the same analyst performed on **10% of the filters** counted, with a test for type II error performed to determine whether a **pair of counts** by the same analyst on the same slide should be **rejected due to non-random fiber distribution**

____ **D.6.3.2(a)** If a pair of counts is rejected by this test, are the **remaining samples** in the set **recounted**, with the new counts tested against the first counts

**Note:** All rejected paired counts shall be discarded

____ **D.6.3.2(b)** Have **all individuals** performing airborne fiber analysis taken the **NIOSH Fiber Counting Course** for sampling & evaluating airborne Asbestos dust, or an equivalent course

____ **D.6.3.2(c)** Has the laboratory participated in a **national sample testing scheme**, such as the Proficiency Analytical Testing (PAT) program or the Asbestos Analysts Registry (AAR) program, both sponsored by AIHA, or equivalent

**Asbestos Calibration**

____ **D.6.5.2(a)** Does the analyst use the **telescope ocular** (or Bertrand lens) supplied by the manufacturer **daily** to ensure that the **phase rings** (annular diaphragm & phase-shifting elements) are **concentric**

____ **D.6.5.2(b)** Is the **phase-shift limit of detection** of the microscope checked **monthly** or after modification or relocation using a **HSE/NPL phase-contrast test slide** for analyst / microscope combination
**Note:** This ensures achievement of a minimum detectable fiber diameter, e.g., <0.25 um

____ **D.6.5.2(c)** Prior to ordering the Walton-Beckett graticule, is calibration performed to obtain a **counting area 100 um in diameter** at the image plane of the microscope
**Note:** The diameter of the circular counting area & the disc diameter must be specified when ordering the graticule

____ **D.6.5.2(c)** Is the microscope **field diameter** checked to a tolerance of **100 +/- 2 um** with a **stage micrometer** upon receipt of the graticule from the manufacturer

____ **D.6.5.2(c)** Is the field diameter **recalibrated to determine the field area** when changes (e.g., zoom adjustment, disassembly, replacement, etc.) occur in the **eyepiece-objective-reticle combination**, or when there is a change in the **interpupillary distance** (i.e., change in analyst)

____ **D.6.5.2(c)** Is the **acceptable range** for the field area **0.00754-0.00817 square millimeters**
**Note:** The actual field area must be documented & used

**Analytical Sensitivity**

____ **D.6.6.2** Is the **normal quantitative working range** 0.04-0.50 fibers per square centimeter for a 1000-L air sample, with the **ideal counting range** on the filter 100-1300 fibers per square millimeter

____ **D.6.6.2** Is the **limit of detection** less than 0.01 fibers per square centimeter for atmospheres free of interferences

**Data Reduction**

____ **D.6.7.2(a)** Is the concentration of Asbestos in a given sample **calculated in accordance with NIOSH 7400,** Issue 2, 15 August 1994, Sections 20 & 21
(refer to NELAC Section 5.5.4.7.2 for additional data reduction requirements)

____ **D.6.7.2(b)** Does the laboratory **calculate & report** intra-laboratory & inter-laboratory standard deviations with **each set of results**

**Quality of Standards & Reagents**

____ **D.6.8.2** Do the **testing reagents & devices** (e.g., the HSE/NPL test slide & the Walton-Beckett graticule) conform to specifications in the test method

## POLARIZED LIGHT MICROSCOPY

**Negative Controls**

____ **D.6.1.3(a)** For **friable materials** is at least **one blank slide** prepared **daily** with every **50 samples** analyzed, whichever is less, by mounting a subsample of an **isotropic verified non-ACM** (non-Asbestiform containing material such as fiberglass in SRM 1866) in a drop of **immersion oil** on a clean slide, rubbing preparation tools in the mount, & placing a clean coverslip on the drop

____ **D.6.1.3(a)** Is the **entire coverslip area scanned** to detect any Asbestos contamination

____ **D.6.1.3(a)** Is a blank slide prepared after every **20 uses** of each piece of **homogenization** equipment, by homogenizing an isotropic verified non-ACM in the clean equipment, a slide prepared with the material, & the slide scanned for Asbestos contamination

____ **D.6.1.3(b)** For **non-friable materials** is at least one non-ACM non-friable material prepared & analyzed with **every 20 samples analyzed**
**Note:** The non-ACM must go through the full preparation & analysis regimen for the type of analysis being performed

**Test Variability / Reproducibility**

____ **D.6.2.1.3** Is **at least 30% of the laboratory's bulk sample QC analyses** performed on samples containing
**D.6.2.3** **1-10% Asbestos**

____ **D.6.2.1.3(a)** Are **at least one out of 50** bulk Asbestos samples **reanalyzed by the same analyst**
**D.6.2.3** **Note:** For single-analyst laboratories, this frequency must be at least one every 10 bulk samples

____ **D.6.2.1.3(b)** Are **at least one out of 15** bulk Asbestos samples **reanalyzed by another analyst**
**D.6.2.3**

____ **D.6.2.1.3(b)** Are **additional reanalyses performed**, possibly including another analyst, to **resolve discrepancies**
**D.6.2.3** when classification errors occur, when asbestos identification errors occur, or when inter-analyst precision is found to be unacceptable

____ **D.6.2.1.3(c)** Does the laboratory **participate in round robin testing** with at least one other laboratory, with
**D.6.2.3** bulk Asbestos samples sent to this laboratory **at least 4 times per year**

____ **D.6.2.1.3(c)** Are the bulk Asbestos round robin samples **previously analyzed as QC samples**, with the results of
**D.6.2.3** round robin analyses **assessed in accordance with QC requirements**
**Note:** As a minimum, the QC requirements must address misclassifications (false positives, false negatives) & misidentification of asbestos types

____ **D.6.3.3(a)** For **friable materials** is at least one out of 100 samples analyzed a **standard or reference sample** that has been routinely resubmitted to determine analyst's precision & accuracy

____ **D.6.3.3(a)** Do at least half of these reference samples contain **between 1-10% Asbestos**

____ **D.6.3.3(b)** For **non-friable materials** is at least one out of 100 samples analyzed a **verified quantitative standard** that has been routinely resubmitted to determine analyst precision & accuracy

**Asbestos Calibration**

____ **D.6.5.3(a)** Is a **properly aligned** polarized light microscope utilized & aligned **before each use**

____ **D.6.5.3(b)** Does the laboratory have & use the **full range** of refractive index liquids **within 1.49-1.72** in intervals of **0.005 or less**, plus refractive index liquids for **dispersion staining**, **high-dispersion series 1.550, 1.605, & 1.680**

____ **D.6.5.3(b)** Are the **refractive index liquids** calibrated at first use & semiannually (or next use if less frequent) to an **accuracy of 0.004**, with a temperature accuracy of **2 degrees Celsius**, by using a refractometer or RI (refractive index) glass beads

**Analytical Sensitivity**

____ **D.6.6.3** Does the laboratory utilize a test method that **provides a limit of detection** appropriate & relevant for the intended use of the data

____ **D.6.6.3** Is the limit of detection **determined by the protocol** in the test method or applicable regulation

**Data Reduction**

____ **D.6.7.3(a)** Is the concentration of Asbestos in a given sample **calculated in accordance with the method** utilized
(refer to NELAC Section 5.5.4.7.2 for additional data reduction requirements)

____ **D.6.7.3(b)** Does the laboratory **determine precision & accuracy** for the percent Asbestos range involved

**Quality of Standards & Reagents**

____ **D.6.8.1(a)** **D.6.8.3** Does the laboratory quality control program **establish & maintain** provisions for **Asbestos standards**

____ **D.6.8.1(a)(1)** **D.6.8.3** Are the **Asbestos reference standards** obtained from NIST, EPA, or suppliers who participate in supplying NIST standards or NIST-traceable Asbestos
**Note:** Any reference standard purchased outside the United States must be traceable to that country's national standards laboratory; commercial suppliers of reference standards must conform to ANSI N42.22 to assure the quality of their products

____ **D.6.8.1(a)(2)** **D.6.8.3** Are these reference standards accompanied with a **certificate of calibration** whose content is as described in ANSI N42.22-1995, Section 8, Certificates

____ **D.6.8.1(b)** **D.6.8.3** Are all reagents used in Asbestos analysis **analytical reagent grade** or better

____ **D.6.8.1(c)** **D.6.8.3** Does the laboratory have mineral fibers or data from mineral fibers that will **allow differentiating Asbestos** from at least the **following look-alikes**: fibrous talc, sepiolite, wollastonite, attapulgite (palygorskite), halloysite, vermiculite scrolls, antigorite, lizardite, pyroxenes, hornblende, richterite, winchite, or any other asbestiform minerals suspected as being present in the sample

COMMENTS:

**EPA 100.1, 100.2; 40 CFR Part 763, App. A to Subpart E**

Transmission Electron Microscope, 80 kV accelerating potential, magnification > 20000
Energy-dispersive X-Ray Analyzer (EDXA)
Vacuum Evaporator, with rotating stage, for carbon deposition onto membrane filter
Polycarbonate Membrane Filters, 0.1-um pore size (EPA 100.1)
Mixed Cellulose Ester Fiber Filters, 0.22-um pore size (EPA 100.2 & 40 CFR Part 763)
Mixed Cellulose Ester Fiber support filters, < 5-um pore size
Electron Microscope Grids, 200-mesh, copper & gold
Chloroform, to dissolve polycarbonate filter
Acetone, or 35% Dimethylformamide/Acetic Acid, to dissolve cellulose fiber filters
Asbestos Standards, NIST-traceable, reference fiber suspension & bulk materials
Interference Minerals (antigorite, attapulgite, halloysite, horneblende, pyroxenes, sepiolite, vermiculite)
NIST Standard Reference Materials (SRM) for Chrysotile unit fibrils, Amosite, & Crocidolite
NIST SRM for Anthophyllite, Tremolite, & Actinolite
Carbon Grating Replica, for TEM magnification calibration
Silicate Mineral Standards (NIST SRM or Riebeckite, Chrysotile, Halloysite, Phlogopite, Wollastonite, & Bustamite), to determine EDXA k-factors of Na, Fe, Mg, Al, & Ca relative to Si
Gold sputtered on carbon-coated TEM grid, to determine the TEM Camera Constant
Aluminum thin film on carbon-coated copper TEM grid, to calibrate EDXA

**40 CFR Part 763, App. E to Subpart E**

Polarized Light Microscope, with polarizer, analyzer, port for wave retardation, graduated rotating stage
Binocular Microscope, 10-45x, for Bulk Asbestos only
Refractive Index Liquids, 1.490-1.570 & 1.590-1.720 in increments of 0.002 or 0.004
Refractive Index Liquids for dispersion staining, 1.550, 1.605, &1.630
Asbestos Reference Samples

**40 CFR Part 763.121, App. A**

Positive Phase Contrast Microscope

COMMENTS: List any test instruments, support equipment, & laboratory work areas that do not meet the above Standards

____ **5.1.1** Does the laboratory fulfill the initial instrument calibration requirements contained in the **mandated test methods & regulations**

EDXA Aluminum K-alpha peak at 1.47-1.49 keV & Copper K-alpha peak at 8.03-8.05 keV (done monthly)
EDXA Resolution of Crocidolite Na K-alpha emission & TEM grid Cu L emission (done monthly)
EDXA Copper K-alpha emission 8.03-8.05 keV (performed each day of use)

COMMENTS: List individual analytes and specific details where the above NELAC Standards are not being fulfilled

____ **5.1.1** Does the laboratory fulfill **additional requirements** specified in the **mandated test methods**

**AHERA & NISTIR Quality Control requirements**

TEM grid opening sizes measured on 20 openings on 20 grids for each lot of 1000 TEM grids received,
OR TEM grid opening size measured on 1 grid opening for each sample
At least 3 TEM grids prepared for each sample
Daily system & alignment checks of the TEM and the eucentric goniometer
Blank analyzed each preparation batch or every 10 samples
Results < 10000 Fibers per Liter for fibers over 10 um length (in water)
Results < 50000 Fibers per Liter (all Asbestos fibers in water)
Results < 18 fibers/mm2 (average) & < 53 fibers/mm2 (maximum for any blank) on TEM grid
(criteria for air sample preparation clean room)
Replicate count of fibers in same TEM grid openings, at least one sample out of 100 analyzed
Duplicate sample analysis for every 100 samples analyzed
Hand-calculation of automated data reduction, or independent hand-recalculation, one sample out of 100 analyzed
Record & verify Selected Area Electron Diffraction (SAED) patterns & X-ray spectrum,
one sample out of every 5 analyzed (micrograph # & spectrum filename recorded on count sheet)
Laboratory Control Sample analyzed (from reference suspension), performed during Analyst training
(Initial Demonstration of Capability) & during comparisons with unknowns
Analysis of SRM, yearly for each Analyst (On-going Demonstration of Capability)
**Note:** Unit Chrysotile fibers visible, tubular structure resolvable, ED patterns easily achieved,
Mg & Si peaks resolved in EDXA spectrum, Na in Crocidolite resolvable

**TEM Grid Acceptance Criteria** (e.g., **EPA 100.2**, 11.23)

70% or more grid openings covered by the replica are intact
The replica is not folded or doubled
The replica is not too dark or display obvious visible filter structure due to incomplete dissolution
The grid is not too heavily loaded to obtain an accurate fiber count
Individual grid openings chosen for counting have < 5% holes or tears in carbon film & < 25% particulate coverage

**SDW Technical Notes & EPA 100.2**, 11.31-11.32 require a sensitivity of 200000 Fibers per Liter for each sample
& an approximately equal # openings analyzed from each of the 3 TEM grids per sample
**Note:** Sensitivity is achieved by filtering sufficient amount of water sample, using a smaller-diameter
membrane filter, & examining more TEM grid openings (**minimum of 4 required**)

**SDW Technical Notes & EPA 100.2**, 11.26 Asbestos Fiber Counting Rules:

Count only fibers with Aspect Ratio > 3:1 (length to width ratio) (>5:1 for air samples under 40 CFR 763)
Bundles counted as 1 fiber as long as aspect ratio is met (maximum length of bundle relative to its mean width)
Count fibers & bundles within clusters (fibers with >2 intersections) or protruding from matrices as 1 fiber,
as long as above criteria for fibers & bundles are met
Count fibers intersecting top & left sides of TEM grid opening (fibers intersecting bottom & right sides not counted)
Count only one end of the fiber (to avoid double-counting)

**SDW Technical Notes & EPA 100.2**, 11.27-11.28 Asbestos Fiber Identification Criteria:

Chrysotile: Examine characteristic SAED diffraction pattern; examine by EDXA if pattern not distinct
Amphibole: Examine SAED for 0.53-nm layer spacing; examine X-ray spectra for elements & peak ratios

**EPA 100.1 & 100.2 Reporting Requirements to Clients** – reports must include:

Mean Concentration of Asbestos, million fibers per liter
Upper & lower 95% confidence limits on the mean Asbestos concentration
Sample Volume used for analysis & dilution factor (if any)
Effective Filter Area
Total Area of filter examined
# of Asbestos structures counted
Analytical Sensitivity
Copy of TEM Count Sheet (if requested)
# of Asbestos structures too complex to classify; # of suspect Chrysotile & Amphibole fibers not positively identified

**EPA 100.2**, 11.33, TEM grids for each sample stored in labeled specimen storage box for 3 years

**Polarized Light Microscopy Identification of Asbestos Fibers**

Morphology & Color
Chrysotile: Wavy fibers, bundles w/ splayed ends & kinks, >10:1 aspect ratio, colorless, nonpleochroic
Amosite: Straight rigid fibers, >10:1 aspect ratio, colorless-brown, nonpleochroic, possible opaque inclusions
Crocidolite: Straight rigid fibers, thick bundles, blue to purple-blue, pleochroic, birefringence generally masked
Anthophyllite: Straight fibers, acicular cleavage fragments, <10:1 aspect ratio, colorless to brown
Tremolite-Actinolite: Acicular-prismatic cleavage fragments, single crystals, <10:1 aspect ratio, colorless-green
Refractive Indices
Chrysotile: alpha 1.493-1.560, gamma 1.517-1.562 parallel to fiber length (normally 1.556)
Amosite: alpha 1.635-1.696, gamma 1.655-1.729 parallel to fiber length (normally 1.696-1.710)
Crocidolite: alpha 1.654-1.701, gamma 1.668-1.717 (normally close to 1.700)
Anthophyllite: alpha 1.596-1.652, gamma 1.615-1.676 parallel to fiber length
Tremolite-Actinolite: alpha 1.599-1.668, gamma 1.622-1.688 parallel to fiber length
Birefringence
Chrysotile: .008
Amosite: .020-.033
Crocidolite: .014-.016
Anthophyllite: .019-.024
Tremolite-Actinolite: .023-.020
Extinction
Chrysotile, Amosite, Crocidolite, Anthophyllite: parallel to fiber length
Tremolite-Actinolite: oblique extinction, 10-20 degrees for fragments, parallel extinction for composite fibers
Sign of Elonation
Chrysotile, Amosite, Anthophyllite, Tremolite-Actinolite: + (length slow)
Crocidolite: - (length fast)
Central Stop Dispersion Staining Colors
Chrysotile (RI Liquid 1.550): Blue (perpendicular), Blue-magenta (parallel)
Amosite (RI Liquid 1.680): Blue-magenta to pale blue (perpendicular), Golden-yellow (parallel)
Amosite (RI Liquid 1.550): Yellow to white (perpendicular), Yellow to white (parallel)
Crocidolite (RI Liquid 1.700): Red magenta (perpendicular), Blue-magenta (parallel)
Crocidolite (RI Liquid 1.550): Yellow to white (perpendicular), Yellow to white (parallel)
Anthophyllite (RI Liquid 1.605): Blue (perpendicular), Gold to gold-magenta (parallel)
Tremolite (RI Liquid 1.605): Pale blue (perpendicular), Gold (parallel)
Actinolite (RI Liquid 1.605): Gold-magenta to blue (perpendicular), Gold (parallel)
Actinolite (RI Liquid 1.630): Magenta (perpendicular), Golden-yellow (parallel)

**Drinking Water Preservation & Holding Time Requirements for Asbestos**

4 degrees Celsius; 48-hr holding time unless samples for ozonated prior to filtration

COMMENTS: List NELAC Standards & Quality Assurance requirements that are not being fulfilled