EMSL Analytical S.O.P.

ISO 10312

Ambient air - Determination of asbestos fibres - Direct-transfer transmission electron microscopy method

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1.0 Method Description

1.1 Applicable Matrix

This method is appropriate for air samples only.

1.2 Scope and application

This method is for the determination of asbestos concentrations in air samples by transmission electron microscopy (TEM); it is applicable to the determination of airborne asbestos in a wide range of ambient air situations both exterior and interior. The range of concentrations which can be determined is 50 structures/mm^2 to 7000 structures/mm2 on the filter.

1.3 Summary of Method

Samples are collected on a membrane filter, prepped via a direct prep method and analyzed with an electron microscope at ≈20,000 X magnification. Asbestos structures are identified by a combination of morphology, elemental chemistry via Energy Dispersive X-Ray Analysis (EDXA), and Selected Area Electron Diffraction (SAED). All structures ≥0.5 micron in length with at least a 5:1 aspect ratio are counted, and recorded. Structures are differentiated as primary or total structures, a primary structure may be composed of numerous total structures. The report includes a breakdown of fibers and bundles > 5 microns as well as true PCM equivalent fibers (>5microns in length, diameter between 0.2 and 3 microns, and a 3:1 aspect ratio). Unless the client specifies otherwise, the lab will attempt to reach an analytical sensitivity of 0.005 structures/cc.

1.4 Detection Limit

The analytical sensitivity is defined as the calculated airborne asbestos structure concentration in asbestos structures per Liter, equivalent to the counting of 1 asbestos structure in the analysis. The analytical sensitivity can be lowered by increasing the volume of air collected and also by increasing the area of the filter analyzed. The Limit of Detection is defined by this method as the upper, one-sided 95% confidence limit of the Poisson distribution for a count of 0 structures. In the absence of background contamination this is equivalent to 2.99 asbestos structures. The limit of detection can be lowered by collecting a larger initial volume of air or by analyzing additional surface area of the filter (grid openings).

2.0 Interferences

Interferences for this method include but are not limited to:

2.1 Non-regulated asbestos minerals such as the two polymorphs of Chrysotile, Lizardite and Antigorite.

2.2 Non regulated amphiboles such as winchite and richterite, and pyroxenes.

2.3 Cleavage fragments of the regulated asbestos types which may at times have aspect ratios similar to the true asbestiform varieties.

2.4 Clay minerals that can have similar morphology to asbestos such as sepiolite and pallygorskite.

2.5 All non-asbestos particulate, fibrous or not which can partially or wholly obscure asbestos fibers.

3.0 Definitions

3.1 Analytical Sensitivity - The airborne concentration represented by one asbestos structure counted under the electron microscope. The air volume collected and the proportion of the filter examined determine the analytical sensitivity. There is no set target A.S. with this method. If not directed otherwise by the client, EMSL will attempt to reach an A.S. of 0.005 s/cc up to 10 G.O. unless the method stopping rule of 100 asbestos structures is reached. A minimum of 4 grid openings must be analyzed.

3.2 Asbestos - Generic term for a group of hydrated mineral silicates

3.3 Aspect Ratio - The ratio of the length to the width of a particle. Minimum aspect ratio for this method is 5:1 (3:1 for optional PCMe phase of analysis).

3.4 Bundle - A grouping of apparently attached parallel fibers. A structure composed of parallel, smaller diameter fibers attached along their lengths. A bundle may exhibit diverging fibers at one or both ends.

3.5 Cluster - A structure in which 2 or more fibers or fiber bundles are randomly oriented in a connected grouping. There are two types of clusters, disperse and compact.

3.6 Compact Cluster - Complex and tightly bound network in which one or both ends of each individual fiber or bundle is (are) obscured, such that the dimensions of individual fibers and bundles cannot be unambiguously determined. As such, no subcomponents or residuals will be listed.

3.7 Compact Matrix - A structure consisting of one or more particles, greater than 0.5 microns in which fibers or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibers and bundles cannot be unambiguously determined. As such, no subcomponents or residuals will be listed.

3.8 Disperse Cluster - A disperse and open network in which at least one of the individual fibers or bundles can be separately identified and its dimensions measured.

3.9 Disperse Matrix - A structure consisting of one or more particle greater than 0.5 microns with overlapping or attached fibers or bundles in which at least one of the individual fibers or bundles can be separately identified and measured.

3.10 EDXA -Energy dispersive X-ray analysis.

3.11 Fiber - An elongated particle which has parallel or stepped sides. For this method it must have a minimum length of 0.5 µm and an aspect ratio of 5:1 or greater.

3.12 Fiber Bundle - See bundle.

3.13 Grid - A thin metal (usually Cu) foil with openings that the sample is mounted on to aid in its examination in the TEM.

3.14 Grid Opening (GO) - One opening of the grid. For this method the area of the grid opening must be known.

3.15 Matrix - One or more fibers or fiber bundles that are attached to or partially concealed by a single particle or group of overlapping non fibrous particles. There are two types of matrices, disperse and compact.

3.16 MCE Filters - Mixed cellulose ester filter.

3.17 PC Filters - Polycarbonate filters.

3.18 PCM Equivalent Fiber - A fiber of aspect ratio 3:1 or greater, longer than 5 microns and a diameter between 0.2 and 3 microns.

3.19 PCM Equivalent Structure - A fibrous structure of aspect ratio 3:1 or greater, longer than 5 microns and a diameter between 0.2 and 3 microns.
3.20 Primary Strucuture - A fibrous structure that is a separate entity in the TEM image and shall be recorded as one of the fundamental structures: fiber, bundle, cluster or matrix.
3.21 Residual - A localized group of fibers that remain after the 5 largest prominent features of a primary structure has be documented. Residuals are named cluster residual (CR) or matrix residual (MR) depending on the identity of the primary structure. Record up to 5 residuals for each primary structure.
3.22 SAED - Selected area electron diffraction.
3.23 Structure - A single fiber, fiber bundle, cluster, or matrix which may contain asbestos.
3.24 TEM - Transmission Electron Microscopy.
3.25 Total Structure - Structure or structures associated with the primary structure. Total structures are enumerated separately from primary structures and are used to classify and measure the important features of each primary structure.

4.0 Safety

All personnel performing preparation and/or analysis of samples must be familiar with the EMSL Chemical Hygiene Plan (EMSLChemHygiene 200.0). Specific hazards and precautions associated with this analysis include:

4.1 Asbestos

4.1.1 Prudent measures must be taken to prevent any possible airborne asbestos fiber release from occurring during sample handling.
4.1.2 Any filter handling performed prior to the filter collapse step should be performed under the safety hood.

4.2 Acetone

4.2.1 Keep away from heat, sparks, and flame.
4.2.2 Avoid breathing vapors - use with adequate ventilation.
4.2.3 Avoid contact with eyes.
4.2.4 Prevent prolonged or repeated contact with skin.

4.3 Carbon Spark

4.3.1 Shield eyes from the glow of the spark during the carbon coating process with welder's type goggles or similar protection.

4.4 Oxygen

4.4.1 The oxygen used with the plasma asher is an explosion hazard. Use only vacuum pumps filled with non hydrocarbon (Fomblin) oil.
4.4.2 Keep Oxygen tank strapped to wall at all times.

5.0 Equipment and Supplies

5.1 ≤0.45 micron MCE or ≤0.4 micron PC filters
5.2 Glass Petri Dishes
5.3 Glass microscope slides
5.4 Low Temperature Plasma Asher
5.5 Vacuum Evaporator (Carbon Coater)
5.6 Graphite or Carbon rods
5.7 HEPA Laminar Flow Hood
5.8 Grids – Copper and Gold
5.9 Fine Forceps

5.10 Grid Clips and Grid Storage Boxes
5.11 Jaffe Wick or Sponge
5.12 Kimwipes or alternative paper
5.13 Transmission Electron Microscope with the following capabilities:
-100 Kev
-fine probe size <250 nm
-Elemental Chemistry via X-Ray Detector

6.0 Reagents and Standards

All reagents should be of recognized analytical grade or better:

6.1 Acetone
6.2 Di-Methyl Formamide (DMF)
6.3 Glacial Acetic Acid
6.4 NIST SRM 1876b
6.5 NIST SRM 2063
6.6 Albite Standard
6.7 Aluminum Coated Copper Grid Standard
6.8 Gold Coated Grid Standard
6.9 Magnification (cross grating replica) Calibration Standard
6.10 NIST Traceable Asbestos on Grids
6.11 Non Asbestos Standards on Grids
6.12 Ethylenediamine
6.13 1-methyl 2-pyrrolidone

7.0 Sample Collection, Preservation, Shipment and Storage

7.1 Samples are collected on ≤ 0.45 micron Mixed Cellulose Ester (MCE) or ≤0.4 micron Polycarbonate (PC) filter cassettes (EMSL recommends MCE filters).
7.2 Air flow should be between 2.4 LPM and 15 LPM (not to exceed 10 LPM recommended)
7.3 Typically 1800 liters or more are collected. The total volume that can be collected will be partially determined by the air being sampled.
7.4 No sample preservation is needed and samples can be stored indefinitely prior to analysis.
7.5 Samples are best transported to the lab by hand. When mailing, try to package samples carefully to minimize disturbance and possible dislocation of particulate from the filter surface. Use packing materials that will minimize static charge.
7.6 All air cassettes must be retained in an easily retrievable manner for a minimum of 60 days.
7.7 All samples prepped to grid (both on the grid clip and in the grid box), will be retained for a minimum of 3 years.

8.0 Calibration and Standardization

Each major component of the method is calibrated and/or standardized including the analyst. Examples follow:

8.1 Sample collection vacuum pumps are calibrated at the beginning and end of a sampling event (with the sample cassette in line) using a rotometer.
8.2 The rotometer in turn needs to be calibrated to a primary standard periodically. (Rotometer use and calibration is the responsibility of the sample collection entity).

8.3 The Electron Microscope is calibrated with various standards for
- **8.3.1** Exact screen magnification at 20,000 X.
- **8.3.2** Exact film magnification at 20,000 X.
- **8.3.3** Spot Size (must be less than 250 nm).

8.4 The X-Ray system attached to the Electron Microscope is calibrated with various standards for:
- **8.4.1** Proper location of Al and Cu peaks.
- **8.4.2** Resolution <175 eV at the Mn peak.
- **8.4.3** Relative Sensitivity (K Factors) determined for various elements.
- **8.4.4** A background-subtracted NaKα integrated peak count rate of more than 1 count per second from a fiber of UICC Crocidolite, 50nm diameter or smaller at an accelerating voltage of 80 kV. The peak to background ratio should exceed 1.0.
  8.4.4.1 The mineral used for calibration of the EDXA system for sodium shall be prepared using a TEM gold grid.

9.0 Procedure

9.1 Sample Receipt

- **9.1.1** Upon receipt of samples, check that the sample information on the Chain of Custody (COC) matches the information on the samples and other paperwork. Any discrepancies must be resolved before proceeding.
- **9.1.2** If the samples do not have a COC then one is completed at time of log in. Have the client fill out the necessary information completely.
- **9.1.3** Information required on the Chain of Custody includes:
  - **9.1.3.1** Client name, address, telephone number, contact person, fax number
  - **9.1.3.2** Project number/ name, state where samples where taken
  - **9.1.3.3** Number of samples sent and sample ID's
  - **9.1.3.4** Type of analysis requested
  - **9.1.3.5** Sample volumes or areas if applicable
  - **9.1.3.6** Turn around time. "RUSH" is not acceptable
  - **9.1.3.7** A date and signature of the person relinquishing the samples
  - **9.1.3.8** All samples MUST be accounted for with the proper sample ID's
  - **9.1.3.9** All samples MUST be sealed, properly bagged and undamaged.
- **9.1.4** All samples must be clocked in at the time of receipt and signed and dated by an EMSL employee. If the lab does not have clock for sample receipt the receiving employee should record the time of receipt also.
- **9.1.5** Check to see if the samples match the COC and if the cassettes are open, damaged, or contaminated. If samples are delivered in the same container or bag as bulk asbestos material samples, if the samples are damaged or if the COC does not match, notify the client.

9.2 Sample Log In

If all of the above criteria for sample receiving are met then the sample can be logged in to Sample Master (LIMS) as per the Sample Master SOP.
- **9.2.1** This process will assign a unique EMSL order number for the project as well as unique lab sample ID's.
- **9.2.2** Sample Master generates an Internal Chain of Custody; however at this time it does not create bench sheets or produce reports. For samples analyzed by ISO

10312, bench sheets and the final report are generated using an EMSL prepared Excel spreadsheet.

9.3 Sample Preparation

9.3.1 ISO 10312 samples may be collected on mixed cellulose ester (MCE) filters (≤0.45 µm) or Polycarbonate (PC) filters (≤0.4 µm).

9.3.2 A direct-transfer preparation is required for this method.

9.3.3 The method specifies that MCE filters are collapsed using a mixture 35 ml DMF / 15 ml Glacial Acetic Acid / 50 ml fiber free DI water. The dissolution of the filters after collapsing may be performed with acetone.

9.3.4 A minimum of 3 grids per sample are prepped and stored.

9.3.5 The specific procedures for direct transfer preparation of the filters are outlined in the Direct Prep of Sample Filters SOP.

9.4 Grid Storage

9.4.1 Grids are stored and location recorded following section 9.5 of EMSL's Direct Transfer Prep SOP.

9.4.2 All ISO 10312 grid clips and grid boxes (analyzed preps and backup grids not mounted on clips, are stored for 3 years or as specified by client/project requirements.

9.5 Sample Preparation Acceptance

9.5.1 Remove the first sample grid clip from the box and insert it into the TEM.

9.5.2 Bring the TEM to a magnification of 300 to 500x and inspect the grids to determine if all of the following conditions are being met.

9.5.2.1 More than 75% of the grid must be covered with replica

9.5.2.2 Grid must have at least 75% intact grid openings.

9.5.2.3 Grids must not have more than 10% opaque area due to incomplete filter dissolution.

9.5.2.4 Total grid area must have <25% overlapping or folded replica.

9.5.2.5 Sample must have <10% overall particulate loading.

9.5.2.6 Particulate loading is uniform from grid opening to grid opening.

9.5.2.7 The fibrous structure loading should be ≤ 7000 str/mm^2.

9.5.2.8 Grid openings selected must not have rips or overlapping folds.

9.5.3 If the samples are judged to be unacceptable due to poor prep quality, reprep the samples as necessary.

9.5.4 If the samples are judged to be overloaded (>10% particulate loading) or if the loading is non-uniform, it may be possible to achieve satisfactory preps and the samples analyzed using ISO 13794. This procedure allows for an indirect transfer preparation and can eliminate the problems of overloading and non-uniform distribution. The client needs to authorize this option if it is deemed appropriate.

9.6 Sample Analysis

9.6.1 At a magnification of 100X, first locate and center the grid to be analyzed using both the specimen selector (located on the left side of the TEM column on top of the left translator where it enters the column) and if present, the grid selector knob located on the specimen arm (see diagram below).

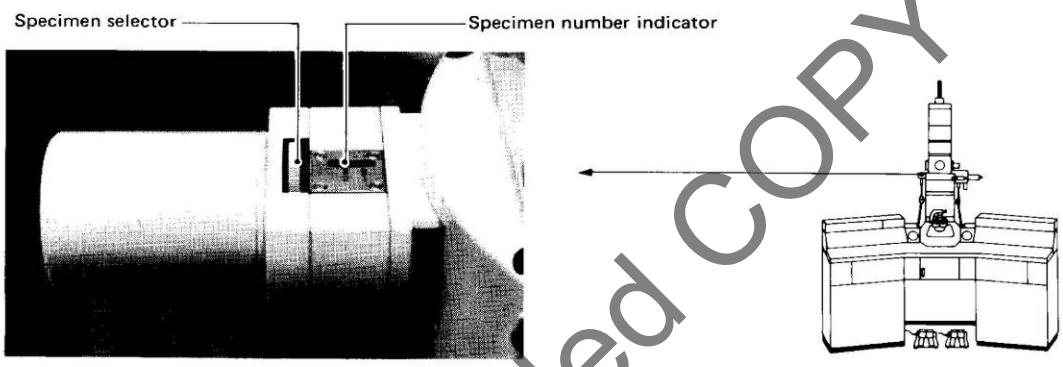

Fig. 4. 17 Specimen selector

9.6.2 The first grid should be located on screen as the grid to the extreme left.

9.6.3 The specimen locator on the scope should be used to find the first grid but only the grid selector knob (if present) located on the specimen arm needs to be employed to move from grid to grid. (Detailed instruction on the use of both long and short clips and their associated sample arms can be found in EMSL's Grid Clip SOP).

9.6.4 Initially, no matter which grid is to be analyzed, you will need to locate grid number 1 and then proceed to the grid of interest (if not grid 1). As analysis proceeds from grid to grid you can simply proceed in the same direction (at 100X) to the next grid.

9.6.5 Next, orient an intact grid opening on the middle of the screen with the left and right stage controls.

9.6.6 Choose an acceptable grid opening to analyze.

9.6.7 Increase magnification to approximately 20,000X taking care to remain in the chosen grid opening.

9.6.8 Log the grid opening identification on the sample worksheet.

9.6.9 Move to the upper left corner of the grid square and begin traversing the grid using only one directional control.

9.6.10 Once the opposite grid bar has been reached, move over/down one field of view using the other directional control (approximately one large circle width ≈ 5µm) and proceed scanning with the original directional controller in the opposite direction.

9.6.11 Repeat this procedure until the entire grid opening has been scanned. Take care not to count any structure twice or to miss any area of the grid opening.

9.6.12 If no fibrous structures have been located , record “ND” for the grid opening and repeat steps 9.6.5 to 9.6.11 until one of the stopping procedures in step 9.6.21 have been reached.

9.6.13 Do not analyze adjacent grid openings. Grid openings selected should be split evenly and randomly between a minimum of 2 grid preps.

9.6.14 Do not count structures that intersect the top and left grid bars. Structures that intersect the bottom and right grid bar are to be counted as twice the length observed extending from the grid bar.

9.6.15 If a fibrous structure has been located, (remember minimum fiber length = 0.5µm with 5:1 aspect ratio) then follow the analysis protocol for each structure encountered as outlined in the flowchart on page 38 of the method for fibers with tubular morphology, and the flowchart on page 41 of the method for fibers without tubular morphology. Both flowcharts are in section 16 of this procedure. Detailed procedures for SAED and EDXA analysis are in their respective EMSL SOPs (SAED SOP and Energy Dispersive X-Ray Analysis SOP).

9.6.16 Record only fibrous Non - Asbestos Minerals (NAM’s) that required close inspection to identify. Enumerate NAM structures with a “0” in the total column and proceed to record all pertinent classifications and distinctions. Both the mineral type and level of ID will be a NAM.

9.6.17 Unless the client specifies otherwise EMSL will attempt to classify Chrysotile to at least a level of “**CD”** (table D.1 page 37 of the method) and Amphiboles to at least “**ADX”** (table D.2 page 37 of the method), both tables are located section 16 of this procedure.

9.6.18 Record at least one SAED pattern on film for each type of asbestos, per sample. Record diffraction information on the sample worksheet. See SAED SOP for detailed information on obtaining and recording SAED patterns.

9.6.19 At least 1 EDXA spectra per asbestos and non-asbestos type, per sample should be printed out or alternately stored to disk as a .pgt file if possible. Record EDXA information on the sample worksheet.

9.6.20 Record all structure information on the bench sheet as follows:

- **9.6.20.1** Primary Structure # Tabulate Primary Structures SEQUENTIALLY
- **9.6.20.2** Total Structure # Tabulate Total Structures SEQUENTIALLY
- **9.6.20.3** Length of all structures, both primary and total.
- **9.6.20.4** Width of all structures, both primary and total.
- **9.6.20.5** Level of ID (for primary structures, this will be the highest level of identification achieved on the associated total structures).
- **9.6.20.6** Mineral Identification
- **9.6.20.7** Sketches and/or comments
- **9.6.20.8** Any photo ID information for negatives taken.
- **9.6.20.9** Whether the EDXA was printed/saved and which structure it corresponds to.

9.6.21 Definitions for the basic structure types are found in the definitions section of this SOP. Example sketches of various structure types are found in the ISO 10312 method and in section16 of this procedure
Record the structure using the following notation.

- **9.6.21.1** Structure types and abbreviations are:
  - **9.6.21.1.1** F - Fiber
  - **9.6.21.1.2** B - Bundle

9.6.21.1.3 CD - Cluster (disperse)
9.6.21.1.4 CF - Cluster Fiber
9.6.21.1.5 CB - Cluster Bundle
9.6.21.1.6 CR - Cluster residual
9.6.21.1.7 CC - Cluster (compact)
9.6.21.1.8 MD - Matrix (disperse)
9.6.21.1.9 MF - Matrix Fiber
9.6.21.1.10 MB - Matrix Bundle
9.6.21.1.11 MR - Matrix Residual
9.6.21.1.12 MC - Matrix (compact)

9.6.21.2 Following all primary structures, except for fibers (F) and bundles (B) are two characters:

9.6.21.2.1 The first character is an estimate of fibers and bundles comprising the structure. A "+" is recorded when there are more than 9 component fibers or bundles within a structure.

9.6.21.2.2 The second character corresponds to the number of component structures described in the first character that are also > 5µm. A "+" is recorded when there are more than 9 subcomponents that are > 5 µm.

9.6.21.3 Record the 5 largest prominent subcomponents, (fibers or bundles), that makeup the structure. Fill in the same information on the bench sheet for these compositional structures as were completed for the primary structures. Do not record more than 5 prominent subcomponents for each primary structure.

9.6.21.4 If, after accounting for prominent component structures, a group of fibers remains, record this as a residual. Do not record more than 5 residuals for each primary structure. A residual is measured and assigned a two digit number derived in the same manner as specified in the primary structure coding above.

9.6.21.5 Compact structures (cluster and matrix) by definition have no sub-components that cannot be isolated and labeled; therefore sub-components or residuals **should not be** recorded.

9.6.21.6 Cluster and Matrix structures can occur in which the characteristics of both types, disperse and compact, happen in the same structure. In instances like this, the structure should be assigned as a disperse structure and a logical procedure should be followed for recording the subcomponents.

9.6. 21.7 When in a matrix, the proportion of the length of a fiber or bundle that is obscured by other particles shall be used as the basis for determining whether it is to be recorded as a separate component. (See flow chart in section 16.7)

9.6.21.7.1 If obscured length could not be more than 1/3rd of the total length, then record that fiber or bundle accordingly, as either a Matrix Fiber (MF) or Matrix Bundle (MB)

9.6.21.7.2 If obscured length could be more than 1/3rd of the total length then the fiber or bundle shall be recorded as Compact Matrix (MC) or a Matrix Residual (MR)

9.6.21.7.2.1 Record as MC if no other subcomponent can be separately identified in the structure.

9.6.21.7.2.2 Record as MR if other subcomponents can be separately identified in the structure.

9.6.21.7.3 If a fiber or bundle crosses a matrix particle (greater than or equal to 0.5 microns) and both ends can be located, the structure should be recorded as a Matrix Fiber (MF) or Matrix Bundle (MB)

9.6.22 Examples of proper syntax follows:

9.6.22.1 **MD31** is a disperse matrix comprised of 3 distinct fibers or structures one of which is greater than 5 microns.

9.6.22.2 **MC30** is a compact matrix estimated to have no more than 3 subcomponents none of which is greater than 5 microns.

9.6.22.3 **CD+1** is a disperse cluster with more than 9 fibers or structures ("+" means more than 9), one greater than 5 microns.

9.6.22.4 **MD+0** is a disperse matrix with more than 9 fibers or structures none greater than 5 microns.

9.6.23 Stopping Criteria

9.6.23.1 Analysis may be terminated after the required analytical sensitivity is achieved, or

9.6.23.2 Analysis may be terminated after the completion of analysis of the grid opening in which the 100th structure was encountered.

9.6.23.3 Regardless of which step above terminates the analytical process, a minimum of 4 grid openings must be analyzed.

9.6.24 Blanks

9.6.24.1 One laboratory blank must be processed with each slide of samples. (Only 1 of the laboratory blanks needs to be analyzed per Order ID.)

9.6.24.2 At least one field blank should be processed with each batch of samples.

9.6.24.3 Filter lot blanks are required at a minimum of 2 for every 100 filters.

9.6.25 There is no method required analytical sensitivity for this procedure; the target analytical sensitivity is set with mutual consent of the client. Unless otherwise specified EMSL will analyzed sample to an analytical sensitivity of 0.005 s/cc.

9.6.26 A Pass / Fail Criteria is not given for this method.

10.0 Calculations

Examples of the calculations are displayed below. Variables include but are not limited to the volume and GOA (grid opening area) which is calculated with every batch of grids.

EFA - Effective filter area of a 25mm cassette = 385mm^2

GOA - Grid opening area (0.00635 mm^2)

N - Number of fibers (If N=0 then default to 1 structure)

V - Volume (1200 liters)

AA - Area analyzed (0.06985 mm^2)
NO - Number of grid openings analyzed (11)
n - Number of Samples
AS - Analytical Sensitivity

10.1 Area analyzed (AA)

$$AA = GOAxNO \qquad AA = 0.00635mm^2 x11 \qquad AA = 0.06985mm^2$$

10.2 Structures per square millimeter (str/mm^2)

$$str/mm^2 = (N/AA) \qquad str/mm^2 = (1/0.06985mm^2) \qquad str/mm^2 = 14.3$$

10.3 Structures per cubic centimeter of air (str/cc)

$$str/cc = \frac{EFAxN}{AAx1000xV} \qquad str/cc = \frac{385mm^2 x5}{0.06985mm^2 x1000x1200L} = 0.023$$

10.4 Analytical Sensitivity (AS)

To calculate the analytical sensitivity, calculate the str/cc using 1 for the number of structures (N) in the formula above.

$$AS = \frac{EFAxN}{AAx1000xV} \qquad AS = \frac{385mm^2 x1}{0.06985mm^2 x1000x1200L} = 0.0046$$

10.5 Grid Openings (NO) required to be analyzed

The number of grid openings (NO) that are required to be analyzed is dependent on grid opening area (GOA), the volume (V) of sample collected and the requested analytical sensitivity (AS) of the analysis.

$$NO = \frac{EFA}{ASxGOAx1000xV} \qquad NO = \frac{385}{0.005x0.00635x1000x1200} = 10.1 = 11$**Important!!**

For this calculation round the result to an integer and ALWAYS round the result UP regardless of rounding rules. In the above example, even though the first decimal place is below 5 (10.1), the result is rounded up to produce 11 required openings.

10.6 Chi Square the test for uniform fiber distribution.

This test is performed to quantify the randomness of the fibers distributed on the filter surface. It is calculated with the following equation:

$$\sum_{i=1}^{i=k} \frac{(n_i - np_i)^2}{np_i}$$

Where (p_i); is the grid opening area divided by the total area of the TEM grid examined. (See10.1 above)

Where n_i is the observed number of primary structures on that grid opening.
Where n is the total number of primary structures observed in the sample.

The value from the above calculation is compared with the significance points of the Chi Square table, having (k-1) degrees of freedom at the 0.001 significance level. Where k is the total number of grid openings analyzed.

11.0 Reporting

The following items are included in the final report (on EMSL letterhead) to the client:

11.1 Asbestos concentration in structures/liter (str/L) categorized as follows

11.1.1 Total asbestos concentration for all asbestos types meeting minimum Identification required, typically CD and ADX

11.1.1.1 For sample where 3 or less structures were counted, report the concentration as less than the corresponding one-sided upper 95% confidence limit for the Poisson distribution.

11.1.1.2 For samples where 4 or greater structures are counted report the mean asbestos structure concentration.

11.1.2 Chrysotile asbestos concentrations are reported as follows:

11.1.2.1 Determine which identification and structure categories will be used to calculate the concentration. This is specified by the client. If it is not specified by the client, use all Total structures identified as CD or better.

11.1.2.2 For sample where 3 or less chrysotile structures were counted, report the concentration as less than the corresponding one-sided upper 95% confidence limit for the Poisson distribution.

11.1.2.3 For samples where 4 or greater structures are counted report both

11.1.2.3.1 The mean chrysotile concentration

11.1.2.3.2 The upper and lower chrysotile concentrations based on the corresponding two-sided Poisson 95% confidence intervals.

11.1.3 Total amphibole asbestos concentrations are reported as follows:

11.1.3.1 Determine which identification and structure categories will be used to calculate the concentration. This is specified by the client. If it is not specified by the client, use all Total structures identified as ADX or better.

11.1.3.2 For samples where 3 or less amphibole structures were counted, report the concentration as less than the corresponding one-sided upper 95% confidence limit for the Poisson distribution.

11.1.3.3 For samples where 4 or greater structures are counted report both

11.1.3.3.1 The mean amphibole concentration

11.1.3.3.2 The upper and lower amphibole concentrations based on the corresponding two-sided Poisson 95% confidence intervals.

11.2 The compositional data for the principle varieties of amphiboles present (if any).

11.3 The analytical sensitivity in str/L.

11.4 The detection limit in str/L.

11.5 Total number of primary structures counted.

11.6 Number of total asbestos structures counted.

11.7 Number of asbestos structures > 5µm.

11.8 Number of asbestos fibers and bundles (combined) > 5µm.

11.9 Number of PCM equivalent asbestos structures.

11.10 Number of PCM equivalent asbestos fibers.
11.11 The level of analysis used when identifying chrysotile and amphibole structures.
11.12 Aspect ratio used to define fibers for the analysis.
11.13 Reference to the Method.
11.14 Client Identification and contact information
11.15 EMSL's order ID.
11.16 Date of receipt of the samples.
11.17 Date of sample collection (if provided).
11.18 Date of the report.
11.19 EMSL and client sample ID numbers.
11.20 Volume of air collected in the sample.
11.21 Effective filter area of the sample media.
11.22 Area of the grid openings used during analysis.
11.23 Microscope magnification used during analysis.
11.24 Initials of the analyst.
11.25 Count sheets are supplied with final results.

12.0 Method Performance

Method performance data can be found in the method Ambient air - Determination of asbestos fibres - Direct-transfer transmission electron microscopy method ISO 10312 First edition 1995-05-01.

12.1 MDL
When no structures are counted the detection limit is considered to be the upper limit of the one-sided Poison confidence interval, or 2.99 structures

12.2 DOC's
Demonstrations of Capability are required for each analytical method.

12.3 PT's
Proficiency tests do not exist for this procedure at the current time.

12.4 Accuracy
Since it is not possible to create a standard filter with a known structure concentration the exact accuracy of the method cannot be determined.

12.5 Precision
The analytical precision is dependant on the number of structures counted and the uniformity of the particulate deposit. Assuming a uniform distribution and structure loading of at least 3.5 structures per grid opening, the coefficient of variance of the counting procedure can be estimated at 10%. It should be noted that in practice, particulate deposits obtained by filtration of ambient air samples are rarely ideally distributed.

13.0 Quality Control

13.1 All QC data must be maintained and available for easy reference and inspection.
13.2 Blanks
13.2.1 Laboratory Blanks: A lab blank is included with each slide of samples prepared. The last slide's lab blank will be prepared for analysis, At least one lab blank for every Order ID should be analyzed.
13.2.2 Field Blanks: at least 1 field blank should be processed with each sample set.

13.2.3 Lot Blanks: A minimum of 2 unused filters from each filter lot of 100 will be analyzed.

13.3 Inter-Analyst QC is at least 4% reanalysis of the same grid openings.

13.4 Intra-Analyst QC is at least 2% reanalysis of the same grid openings.

13.5 Verified analysis is used for training and will be performed as need for discordant sample QC reconciliation.

13.6 0.5% repreparation and analysis of sample filter QC.

13.7 Inter-laboratory analysis is performed in order to monitor systematic errors among microscopists as needed or as determined by client.

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14.0 Data Assessment

14.1 Acceptance criteria for QC measures

These are addressed in the EMSL's QA Manual Module A section A.12.6.2

14.1.1 If a sample falls outside the acceptable limits it needs to be reconciled with participating analysts and/or a third analyst when necessary.

14.1.2 The Pass/Fail criteria for repeat results (inter and intra analyst) should not differ at the 5% significance level.

14.1.3 For Verified Analysis, the results should be *≤ 80 % of true positives, ≤ 20% false negatives, and ≤ 10 % false positives* **of** the Total Structure count.

14.1.4 Repreparations from different sectors of the filter is a test for the reproducibility of the whole method. Since this type of QC is impacted by circumstances outside the control of the analysis, no statistical evaluation is currently performed on this data. The data is simply compiled for informational purposes only.

14.1.5 Criteria for the maximum allowable contamination levels for laboratory blanks: on all filter types (MCE and PC) Filters, cannot exceed the methods limit for lot blanks, which is 10 str/mm², or if the mean fiber count for asbestsos fibers and bundles longer than 5 microns is more than 0.1 fiber/mm². .

14.1.6 The Chi Square uniformity test shall be conducted using the number of primary structures found on individual grid openings. (See section 10.6 of this SOP) This value will be compared with significance points of the Chi Square distribution. If the structure count fails this test, the precision of the result may be in question and if new air samples cannot be collected, additional grid openings may need to be examined or alternatively, the sample should be prepared indirectly by ISO 13794.

14.2 Corrective actions

These policies are addressed fully in the EMSL's QA manual section 18.

14.2.1 All corrective actions should look for the root cause of the error.

14.2.2 All out of control or unacceptable data must be brought to the attention of the Laboratory Manager.

14.2.3 The Laboratory manager is responsible for generating a corrective action including an investigation of calibration procedures, a review of analytical technique and investigation of training policies and compliance.

14.2.4 Corrective actions will be reported to the QA Department by means of the Quarterly Management Report or sooner when appropriate.

14.3 Contingencies for handling out-of control or unacceptable data.

Any quality control requirements not met must have an explanation to their Non-conformance.

15.0 Pollution Prevention / Waste Management

15.1 Pollution Prevention

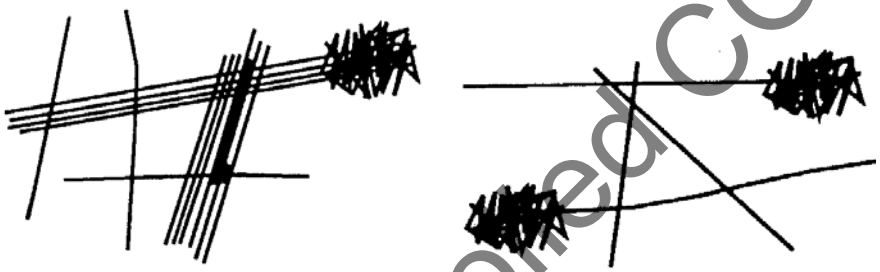
EMSL Analytical makes all efforts to reduce the volume and toxicity of the waste generated by the laboratory. An effort to manage procurement of hazardous materials has been implemented in order to avoid over ordering. Hazardous waste is classified for proper disposal.

15.2 Waste Management

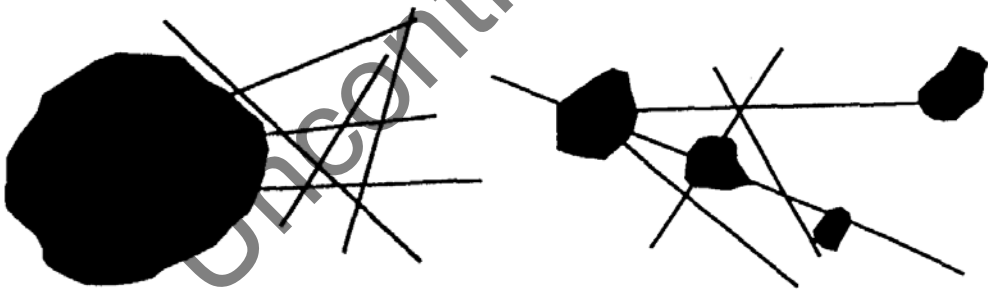
The waste generated during prep and analysis will be disposed of following safety procedures outlined in the chemical hygiene plan (EMSLChemHygiene 200.0).

16.0 Tables, Diagrams, Flowcharts, and Validation Data

16.1 Example of disperse clusters:

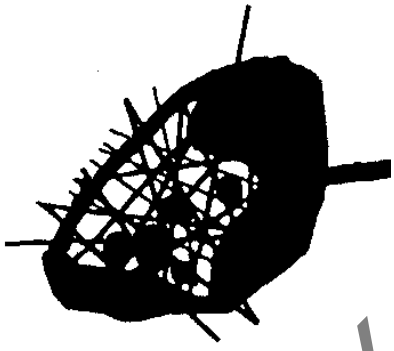


16.2 Examples of disperse matrices:



16.3 Examples of compact Cluster (a) and Matrix (b):

(a)



(b)

16.4 Structure Classification Tables

Table D.1 — Classification of fibres with tubular morphology

Category	Description
TM	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	Characteristic Chrysotile Morphology
CD	Chrysotile SAED pattern
CQ	Chrysotile composition by Quantitative EDXA
CMQ	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	Non-Asbestos Mineral

Table D.2 — Classification of fibres without tubular morphology

Category	Description
UF	Unidentified Fibre
AD	Amphibole by random orientation SAED (shows layer pattern of 0,53 nm spacing)
AX	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	Amphibole by random orientation SAED and qualitative EDXA
AQ	Amphibole by Quantitative EDXA
AZ	Amphibole by one Zone-axis SAED pattern
ADQ	Amphibole by random orientation SAED and Quantitative EDXA
AZQ	Amphibole by one Zone-axis SAED pattern and Quantitative EDXA
AZZ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle
AZZQ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle, and Quantitative EDXA
NAM	Non-Asbestos Mineral

16.5 Identification Flowchart for fibers with Tubular Morphology

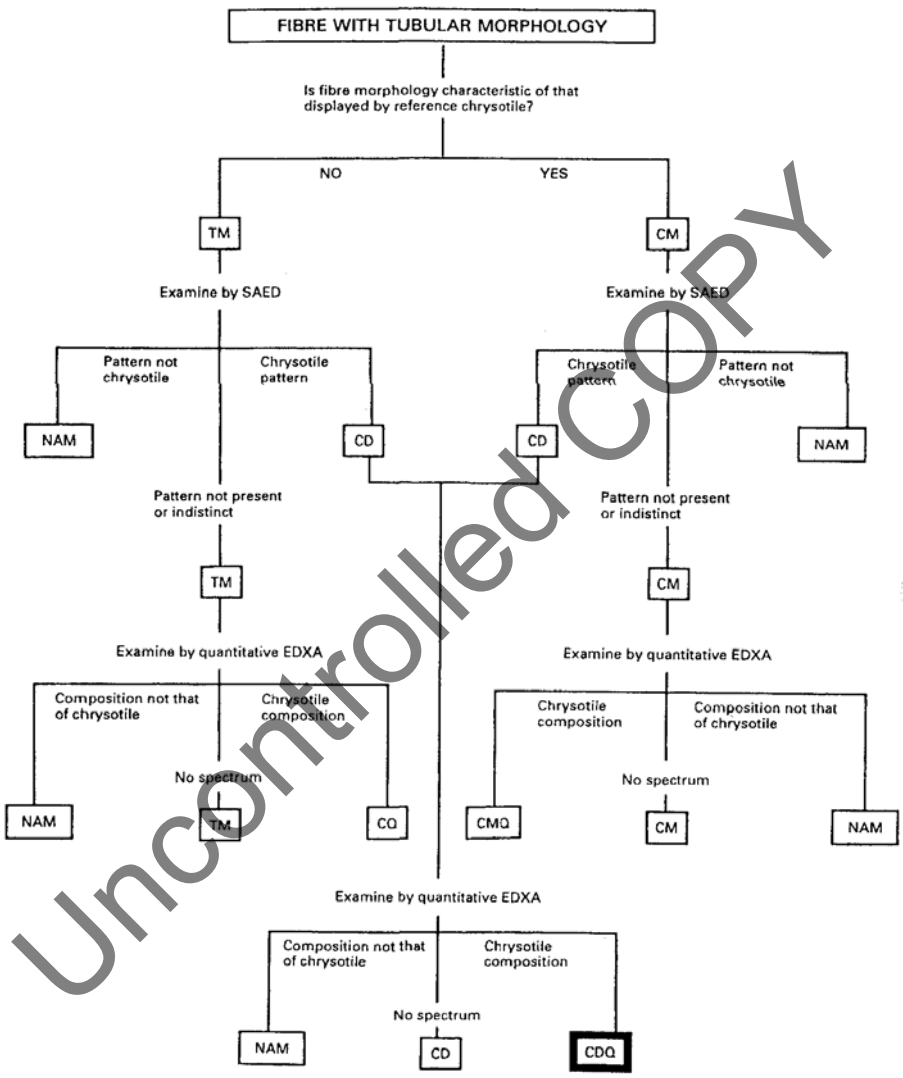


Figure D.2 — Classification chart for fibre with tubular morphology

16.6 Identification Flowchart for fibers without Tubular Morphology

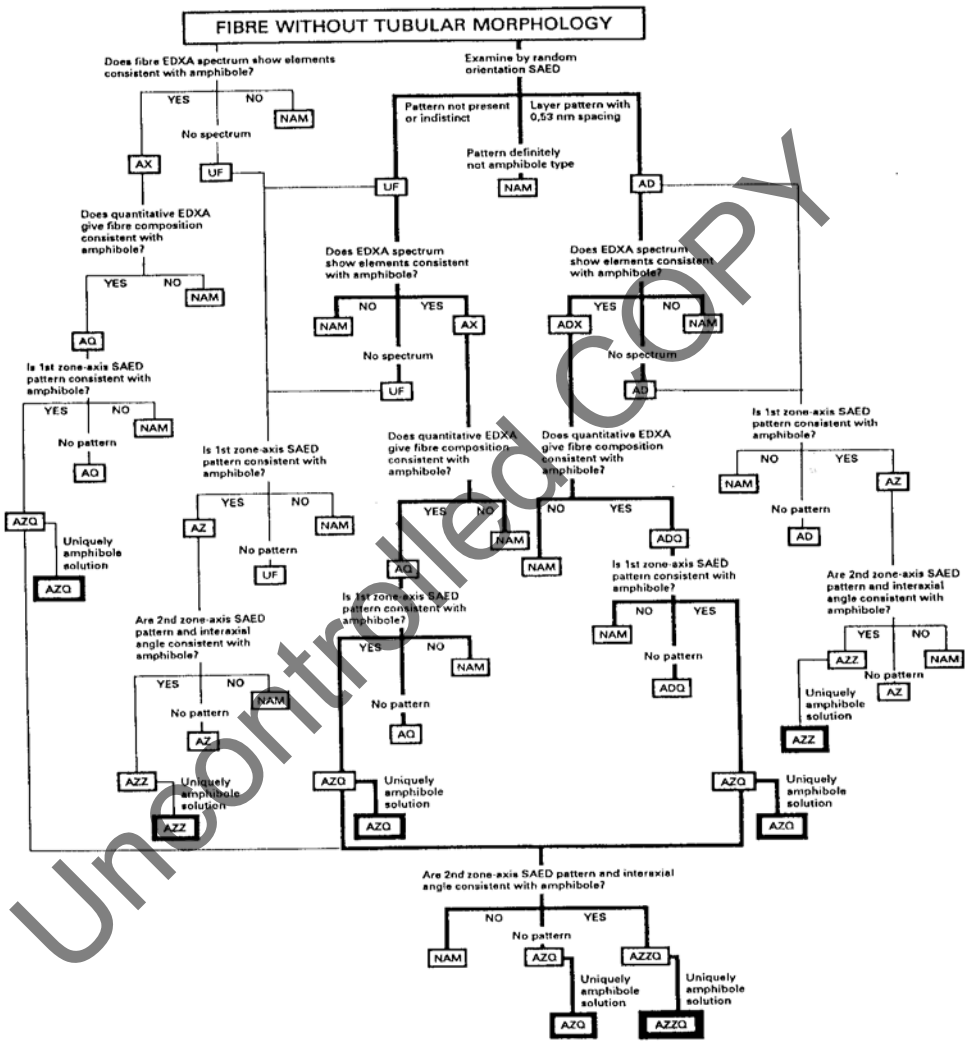
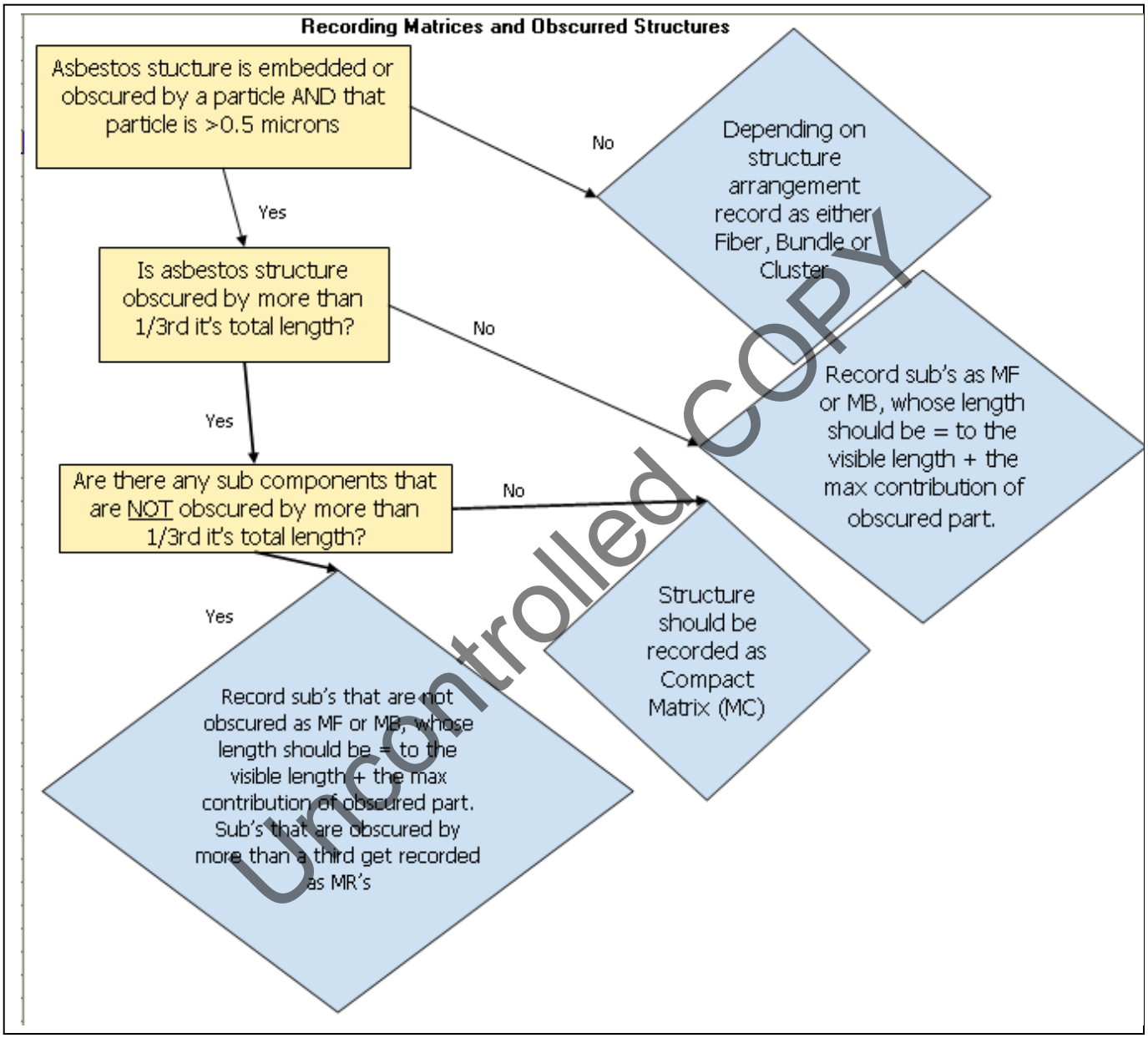


Figure D.4 — Classification chart for fibre without tubular morphology

16.7 Flow chart for recording partially obscured fiber and bundles



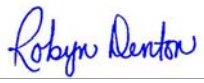
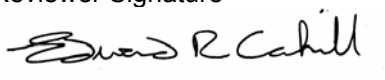
17.0 References

17.1 Ambient air - Determination of asbestos fibres - Direct-transfer transmission electron microscopy method ISO 10312 First edition 1995- 05- 01.
17.2 Less is Better- Guide to Minimizing Waste in Laboratories prepared by the Task Force on
17.3 Laboratory Environment, Health and Safety- American Chemical Society 2002.
17.4 EMSL QA 101.7 Revision 7, September 2004– EMSL Quality Assurance Manual
17.5 EMSL QA Manual Revision 9 April 2007
17.6 EMSL Chemical Hygiene Plan Revision 0, September 2004

18.0 Revision History

Revision #	Date	Revision	Initials
7	01/23/08	Edited for content and NELAC compliance.	KN
8	04/17/08	Corrected syntax description in step 9.6.22.3, original was incorrect for example syntax.	KN
8.1	01/19/09	Reformatted to conform to newer SOP format. Grid storage and recording changed to refer to EMSL Direct Prep SOP.	KN
9.0	02/06/12	Revised section 8.4 (Calibration and Standardization) to include Na sensitivity requirements. Revised section 10.6 to include the chi square test. Revised section 11 (Reporting) to provide a statement as to which structure category and identification category to include in the calculation of the asbestos concentration. Revised section 13 (Quality Control) and section 14 (Data acceptance) to conform to the quality assurance measurements defined in the method. Revised section 16 to include flow chart detailing the recording of partially obscured fibers or bundles. Revised Blank QC determinations	RD
9.1	02/16/12	Revision of section 1.3: definition of a True PCMe fiber.	RD

Authorizing Signatures

Robyn Denton		02/16/2012
Author (Print)	Author Signature	Date
Ed Cahill		02/16/2012
Reviewer (Print)	Reviewer Signature	Date
Ed Cahill		02/16/2012
Corporate Approval (Print)	Corporate Approval Signature	Date

SOP Review and Acknowledgement Form

Instructions: Ensure all personnel in laboratory affected by this procedure read and acknowledge. Once completed, the page shall be retained in an Acknowledgments binder along with personnel training.

Laboratory Manager Acknowledgement

My signature below signifies that I have read and understand the entire contents of this document. My signature represents that I agree to fully comply with, implement, and enforce all requirements, procedures, and protocols specified in these procedures set forth in this document and any supporting reference materials or methodologies.

Laboratory Manager Signature _____ Date _____

Laboratory Staff Acknowledgement

My signature below signifies that I have read and understand the entire contents of this document. My signature represents that I agree to fully comply with, implement, and enforce all requirements, procedures, and protocols specified in these procedures set forth in this document and any supporting reference materials or methodologies.

Signature	Date