National Institute of Standards & Technology

Certificate

Standard Reference Material 1876b

A Chrysotile Asbestos Standard Reference Material for Transmission Electron Microscopy

This Standard Reference Material (SRM) is intended primarily for use in evaluating the techniques used to count and identify chrysotile asbestos fibers in filter samples by transmission electron microscopy (TEM). It can be used as a quality assurance and training material to analyze filter material by the Environmental Protection Agency (EPA) method for the measurement of asbestos in air [1]. The National Institute of Standards and Technology (NIST) modifications to the EPA method are given in the Appendix to this Certificate.

SRM 1876b consists of ten 3 mm x 3 mm sections of 0.45 μm mixed-cellulose-ester (MCE) filters containing chrysotile fibers deposited by an aerosol generator. Sections labelled from one to ten are mounted on two glass slides (one slide contains filter sections #1 through #5, the other slide contains filter sections #6 through #10).

The loading of each SRM unit is 18.2 ± 3.5 structures per 0.01 mm^2 of filter area where "structure" is the countable unit of asbestos as defined by EPA and the attached counting rules. The certified value was determined by using the NIST "verified counting" procedure and represents the best estimate of the true number of structures per 0.01 mm^2 [2].

The certified value is a trimmed-mean count derived from 12 independent counts each normalized to an area of 0.01 mm^2. The trimmed-mean value is obtained by averaging ten counts after the highest and lowest counts are eliminated from the 12 counts. Note: A 95% prediction interval for the trimmed mean is from 14.7 to 21.7 structures per 0.01 mm^2 [3,4]. A laboratory making 12 independent measurements of fiber loadings on grid openings approximately 0.01 mm^2 in size, discarding the lowest and highest values obtained, and computing the arithmetic average of the remaining 10 counts, should expect, with 95% confidence, to find that average within the specified interval.

The measurements were performed by S. Turner, J.M. Phelps, E.S. Windsor, and R.L. Myklebust in the Surface and Microanalysis Science Division of NIST. Measurements were also performed by S.S. Doorn and S.B. Burris of the Center for Environmental Measurements and Quality Assurance, Research Triangle Institute.

Statistical analysis of the certification data was performed by S.D. Leigh of the NIST Statistical Engineering Division.

Fiber deposition on the MCE filters was performed by H. Parish at the Stanford Research Institute.

The overall direction and coordination of the measurements leading to certification were performed under the direction of E.B. Steel and R.A. Velapoldi of the NIST Surface and Microanalysis Science Division.

Support for measurement research and standard development was provided by the Quality Assurance Division, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency.

The technical and support aspects involved in the certification and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by N.M. Trahey.

Gaithersburg, MD 20899
January 9, 1992

William P. Reed, Chief
Standard Reference Materials Program

(over)

Preparation of Filter Sections

In general, the preparation of the filter sections should follow the EPA method [1] with the exception that *no ashing of the sections should be performed.* To compare results with the certified value for SRM 1876b, the user should prepare six of the ten filter sections for measurement, reserving the other four sections as backup. All ten sections should *not* be prepared at the same time in the event there is an unforeseen problem with a preparation run. The number assigned to the filter section (numbers 1-10 on the glass slides of the SRM) should be retained for the prepared filter section and referenced in any consultation with NIST personnel. To aid users of this SRM, the nonloaded side of the filter sections have been marked with a blue dye (methylene blue). The area of the grid squares in the grids should be checked *prior* to preparation of the filter squares. The grid squares counted for this SRM must have an area that is at a minimum 8000 μm^2.

In order to become accustomed to the small size of the sections, it is strongly recommended that users of this SRM practice filter preparation procedures on 3 mm x 3 mm sections of other 0.45 μm MCE filters before preparing sections of this SRM.

The variables that can adversely affect the quality of a replica preparation are not fully understood at this time. It is suspected that environmental factors such as room temperature and humidity can affect the quality of the preparation. The procedure given below has been successfully used to prepare filters at NIST, but may have to be modified for preparation in another laboratory. Materials and reagents used at NIST for preparation of the SRM filter sections include the following[a]:

1) Anticapillary forceps	- for removing sections from a Jaffe wick (optionally, fine forceps can be used)
2) Fine forceps	- for removing filter sections from SRM glass slides
3) Scalpel	- for removing collapsed filter sections from slides
4) Cleaned glass slides	
5) Micropipette	- for measuring 0.5 μL of collapsing solution
6) Oven or slide warmer	
7) Jaffe wicks	- consists of a glass jar with a screen covered by a lens-paper wick
8) Lens paper	
9) Collapsing solution	- 50% dimethyl formamide (DMF) and 50% deionized water by volume
10) Acetone	
11) 200-mesh TEM grids	

Procedure:

1) Remove one of the two slides from the styrofoam box in a clean facility as specified in the EPA method[1]. Check the filter sections by looking at the back of the slides for the presence of a blue marking on the filter sections. (The blue marking indicates the sides of the filter sections that are not loaded with chrysotile). Select 6 sections and note the number(s) (1-10) under the filter sections to be prepared; assign the same number(s) to the grids prepared from the filter sections.

2) Place a 0.5 μL drop of collapsing solution on a clean slide. (This amount of solution will be totally absorbed by the filter section. Use of more than this amount will hinder sample preparation.)

3) Using a pair of fine forceps, remove a filter section from its slide and place it in the collapsing solution at approximately a 45° angle and then release it, so that air is not trapped under the filter section.

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[a]These materials and reagents should be checked for asbestos contamination prior to use.

4) Place the prepared sample slide in an oven or on a slide warmer set at 65 - 70 °C for one hour to dry the filter section.

5) Coat the collapsed filter section with less than 50nm (500 Å).

6) Gently remove the collapsed and coated section from the slide by carefully sliding a scalpel under it. When the section is almost totally detached from the slide, use forceps to finish the separation process (to prevent the section from "popping" off the slide).

7) Place the section on a 200-mesh TEM grid in a Jaffe wick containing DMF. Leave it there for 12 hours to ensure that all of the filter is dissolved away.

8) Remove the grid containing the filter from the Jaffe wick using anticapillary forceps and place it into a Jaffe wick containing acetone for one hour. The acetone vapor acts to clear the filter of any artifacts due to the DMF solution.

9) Remove the grid from the Jaffe wick and check it optically for the quality of the filter preparation.

Counting the Fibers

The user should randomly select two nonadjacent grid openings that are about 0.01 mm^2 in area from each of the six filter sections prepared (minimum area of grid openings must be 0.008mm^2). Counting and identification of the chrysotile fibers should then follow the protocol in the EPA method with the clarifications and modifications to the counting rules given in the Appendix [1]. The user should compute a trimmed-mean value by eliminating the highest and lowest counts obtained in the 12 grid openings, and determining the average of the remaining counts. The trimmed-mean value should be expressed as the number of structures per 0.01 mm^2.

REFERENCES

[1] Code of Federal Regulations, Asbestos-containing materials in schools; final rule and notice, 40 CFR Part 763, 41826-41905, 1987.

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

[3] G.J. Hahn and W. Nelson, A survey of prediction intervals and their applications, Journal of Quality Technology, 5, No. 4, 178-188, 1973.

[4] J.K. Patel, Prediction intervals-a review, communications in statistics: Theory and Methods, 18, No. 7, 2393-2465, 1989.

Appendix to SRM Certificate
Standard Reference Material 1876b

The following information is supplied for the convenience of the user of this Standard Reference Material.

Descriptive Information

The counting rules provide the methodology for determining the value assigned for the number of fibers in an arrangement of asbestos. The counting rules are from two sources. Those in section I contain illustrations of the counting rules given by the EPA [1]. Those in section II contain illustrations of clarifications and modifications to the EPA rules developed by NIST.

Counting Rules Used for Analysis

Counting rules specify how an asbestos fiber or arrangement of fibers will be counted. This Appendix contains illustrations of the counting rules used to determine the quantity of asbestos present on the filter material used in this SRM. This Appendix is included for several reasons. Firstly, several sets of rules have been used or proposed in the past and there will likely be other sets of rules used in the future. This Appendix provides a record of the rules used for analysis of this SRM. Secondly, the counting rules used can have a significant impact on the amount of asbestos determined in an analysis. The certified value given in this SRM applies only to analyses done using the counting rules of this Appendix. Thirdly, the basic rules used for analysis are those provided by the EPA [1] given in section I of the Appendix. However, NIST has found it necessary to modify and clarify the rules to achieve sufficient precision for certification. These modifications and clarifications have not been previously published and are given in section II of the Appendix. For analysis of the SRM, the user should use the rules as modified and clarified by NIST (section II).

Section I. EPA Counting Guidelines for Determining Asbestos Structures

The following definitions relate to classification of asbestos structures:

1. "Fiber" – A structure having a minimum length greater than or equal to 0.5 μm and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides.

2. "Bundle" – A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

3. "Cluster" – A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group. Groupings must have more than two intersections.

4. "Matrix" – Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

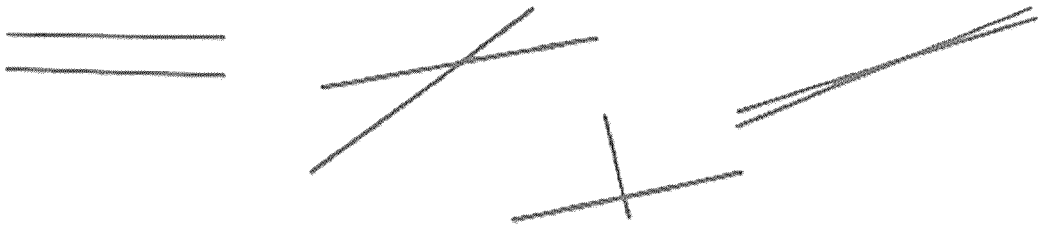
5. "Intersection" – Nonparallel touching or crossing of fibers, with the projection having an aspect ratio of 5:1 or greater.

Examples of the EPA counting rules are given in Figure 1.

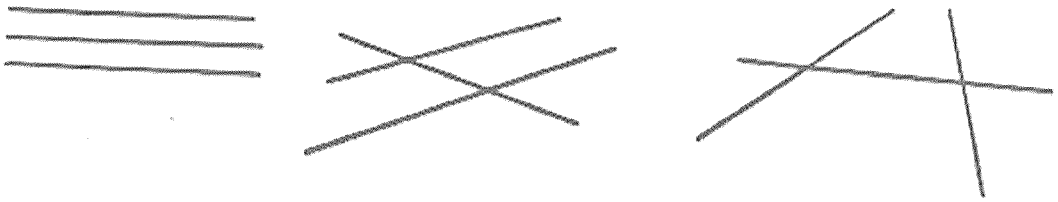
Figure 1. EPA counting guidelines to determine asbestos structures

Count as 1 fiber: 1 structure; no intersections. (Clarified by NIST, see text on pg. 7 and Fig. 2)

Count as 2 fibers if space between fibers is greater than width of 1 fiber diameter or number of intersections is equal to or less than 1. (Modified by NIST, see text on pg. 7 and Fig. 3a; text on pg. 8 and Fig. 7)



Count as 3 structures if space between fibers is greater than width of 1 fiber diameter or if the number of intersections is equal to or less than 2. (Modified by NIST, text on pg. 7 and Fig. 3a; text on pg. 8 and Fig. 7)



Count bundles as 1 structure; 3 or more parallel fibrils less than 1 fiber diameter separation. (Modified by NIST, text on pg. 7 and Figs. 3, 5)

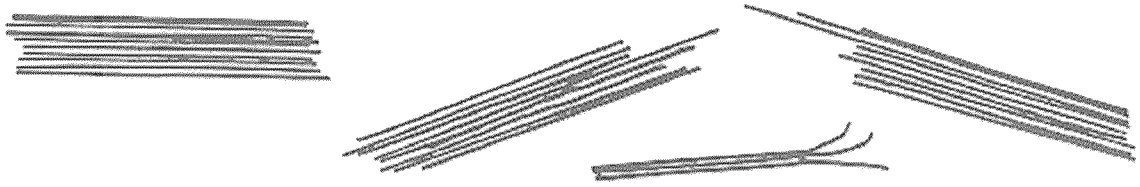
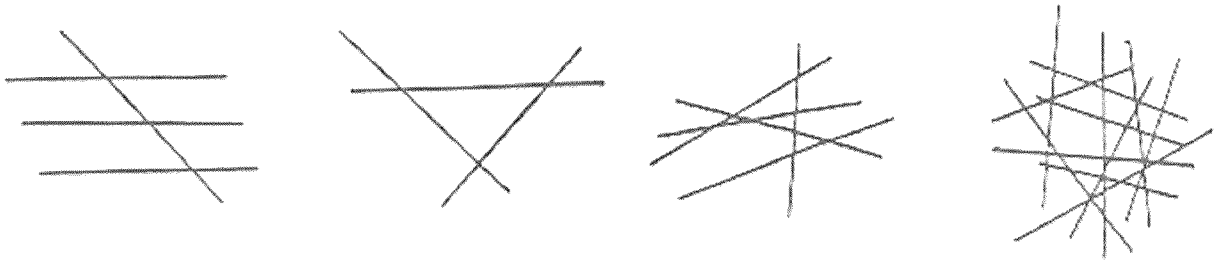
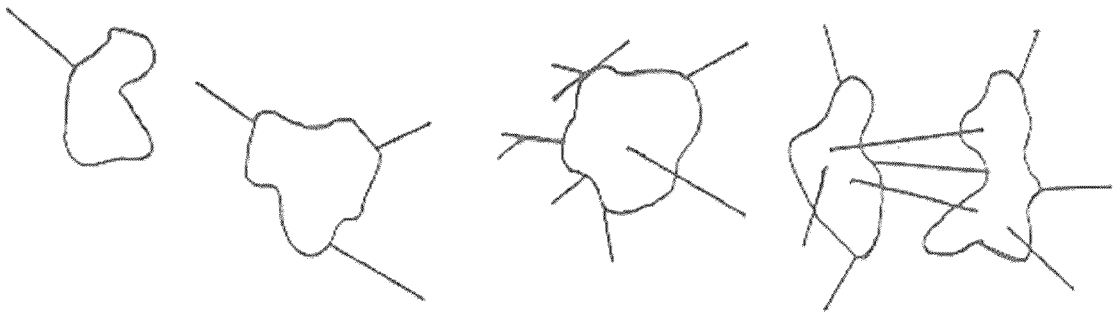


Figure 1. (continued)

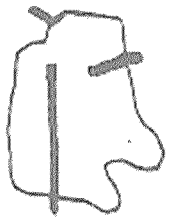
Count clusters as 1 structure; fibers having greater than or equal to 3 intersections. (Clarified by NIST text on pg. 7 and Figs. 4, 5)



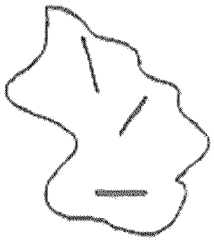
Count matrix as 1 structure. (Modified and clarified by NIST text on pg. 7 and Fig. 6)



Do not count as structures:

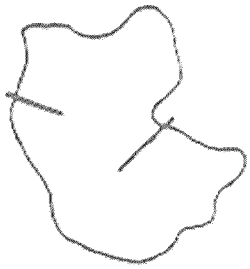


Fiber protrusion
<5:1 Aspect Ratio



No fiber protusion

<0.5 micrometer in length
<5:1 Aspect Ratio



Fiber protrusion
<0.5 micrometer

Section II. NIST Clarifications and Modifications to the EPA Counting Guidelines

The following definitions are the NIST definitions for the counting rules by which the SRM was characterized. In some cases, clarifications and modifications are made to the EPA counting guidelines (section I of this Appendix). The reasons for modifications are indicated.

1. "Fiber" – A structure having a minimum length ≥ 0.5 μm and an aspect ratio (length to width) ≥ 5:1 and substantially parallel sides.

   Clarification of the EPA definition: If the fiber has irregular terminations, the length is defined the longest dimension parallel to the fiber axis - see Figure 2.

2. "Bundle" – A structure composed of two or more fibers that are parallel and touching.

   Modifications to the EPA definition: 1) a bundle is defined as two or more fibers rather than three or more fibers. 2) the fibers must be parallel and touching rather than within a fiber's width - see Figure 3a.

   Reasons for modifications: In the EPA rules, 1) two or more parallel or touching fibers are not defined as a structure (neither as a fiber nor as a bundle), 2) it is not clear which fiber width would be used as a criteria for determining separation of fibers.

   Clarifications of the EPA definition: Those bundles that have splayed ends, that are bent and those that contain fibers that have bowed away from the main bundle are counted as one bundle. Examples are given in Figure 3b.

3. "Cluster" – A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group. Groupings must have more than two intersections.

   Clarification of the EPA definition: If three or more fibers intersect at the same place in a structure arrangement, it is counted as three intersections and therefore as a cluster. Examples are given in Figure 4.

   Clarifications that apply to both bundles and clusters: 1) For a bundle or cluster to be countable, a fiber within the structure must have an aspect ratio ≥ 5:1 and be ≥ 0.5 μm in length. Examples are given in Figure 5a. 2) A "noncountable" asbestos structure is defined as an asbestos fiber that is < 0.5 μm and/or has an aspect ratio < 5:1. If a "noncountable" asbestos structure is touching and parallel to a countable bundle or fiber, it is treated as if it were a countable structure. If the "noncountable" asbestos structure is touching but not parallel to a countable bundle or fiber, it is ignored. Examples are given in Figure 5b.

4. "Matrix" – Fiber or fibers with one end free and the other end embedded in or hidden by a particulate that is ≥ 0.5 μm in its longest dimension.

   Modification to the EPA definition: The EPA rulings have no size constraints on the size of the nonasbestos particulate. The NIST rules consider particles that are < 0.5 μm in their longest dimension to be insignificant and they do not qualify as matrices. Examples of counting asbestos structures associated with particles are given in Figure 6a.

   Clarifications of the EPA definition: 1) If a fiber or bundle is touching a nonasbestos particle so that both ends are free, the fiber or bundle is counted as one structure (fiber or bundle). For examples, see Figure 6b. 2) If a fiber or bundle has both ends in nonasbestos particles, the fiber or bundle counts as one structure (matrix). For example, see Figure 6b.

5. "Intersection" – A single intersection is defined as the nonparallel touching or crossing of two fibers, two bundles or a fiber and a bundle.

   Modifications to the EPA definition: 1) the NIST definition includes bundles whereas the EPA definition relates only to fibers - see Figure 7a, 2) the NIST definition does not specify information about "projections" whereas the EPA definition specifies that "the projection" has an aspect ratio ≥ 5:1 - see Figure 7b.

   Reasons for modifications: In the EPA rules, 1) there was an ambiguity in interpretation of intersecting bundles and 2) the meaning of the term "the projection" is not clear.

Additional Ruling (not covered in the EPA rules)

Fibers, bundles, clusters on grid bars – A fiber, bundle or cluster touching a grid bar is counted the same way it would be if the grid bar was not there. Examples are given in Figure 8.

Figure 2. Fibers

If a fiber to be measured has irregular terminations, the length is defined as the longest dimension parallel to the fiber axis as indicated in the two cases above.

Figure 3 - Bundles

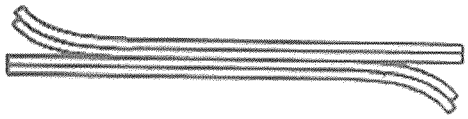
Figure 3a. Closely spaced, parallel bundles and fibers

Count the cases on this row as 2 structures. If there is any space between parallel fibers or bundles, they are counted as separate structures.

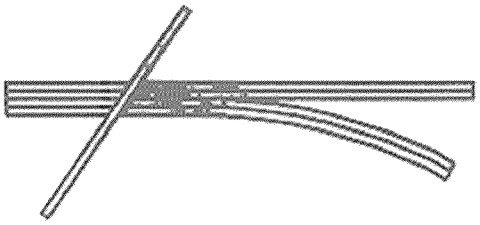
Figure 3b. Nonstandard bundles:

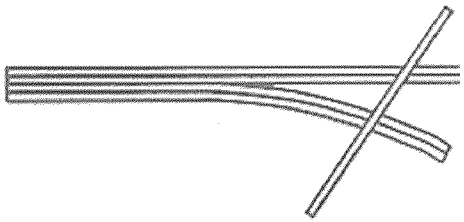
Count as 1 structure (1B)
(bowed bundle)

Count as 1 structure (1B)
(bent bundle)



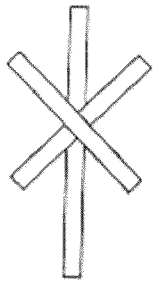
Count each in this row as 1 structure (1B)
(splayed bundles)



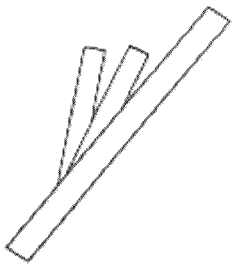


Count each arrangement in this row as 2 structures (1B, 1F)
single fiber intersecting splayed bundle)

Figure 4. Clusters



1 structure (1C)



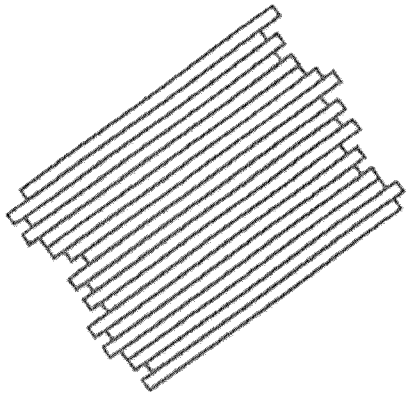
1 structure (1C)

If three or more fibers intersect at the same place, they are counted as three or more intersections and therefore as a cluster.

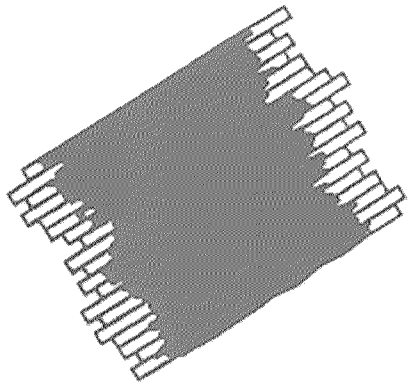
Figure 5. Further clarifications to rules for bundles and clusters.

Figure 5a. Determining countable bundles and clusters.

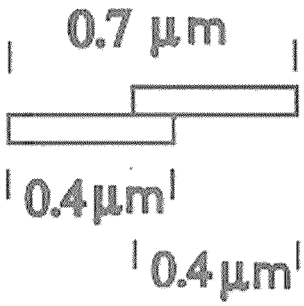
For a bundle or cluster to be countable, a fiber within the structure must have a ration of 5:1 or greater and be longer than 0.5 µm. The overall ratio of dimensions of the bundle or cluster or its length are not relevant to determining a countable structure.



Count as 1 structure
(contains fibers ≥ 0.5 µm,
fibers ≥ 5:1 ratio)

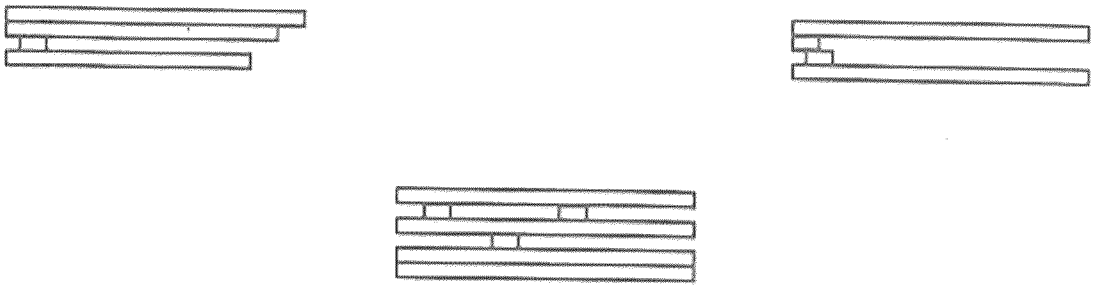


Count as 0 structures
(cannot distinguish
fiber ≥ 0.5 µm, ≥ 5:1 ratio)



Count as 0 structures
(does not have fiber ≥0.5 µm)

Figure 5b. Bundles and clusters containing noncountable asbestos structures.



Count all cases in this row as 1 structure (1B)
(noncountable asbestos touching and parallel to countable asbestos)

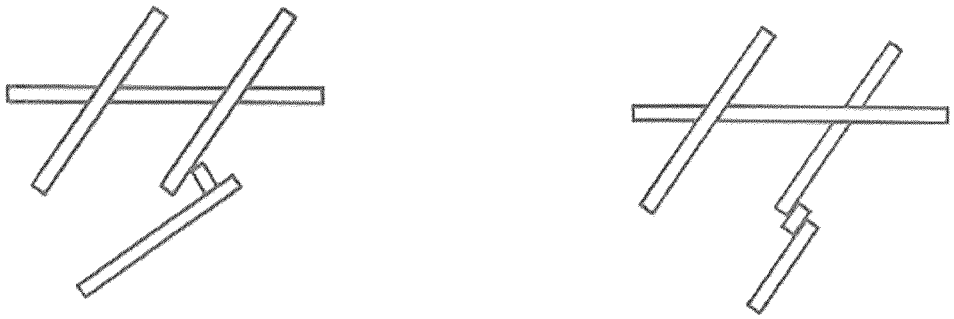
2F

Count as 2 structures

1F

Count as 1 structure

(noncountable asbestos touching but not parallel to countable asbestos)



4F

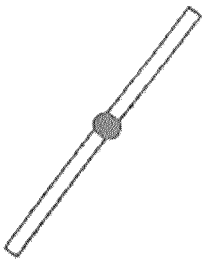
Count as 4 structures

1B, 2F

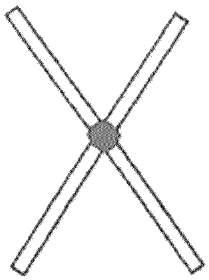
Count as 3 structures

Figure 6. Matrices

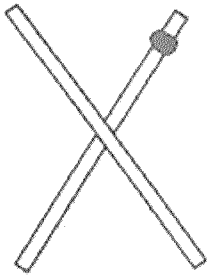
Figure 6a. Asbestos associated with nonasbestos particles.



1 structure (1F)

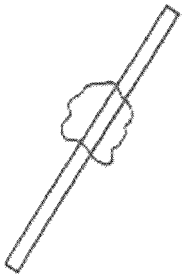


2 structures (2F)

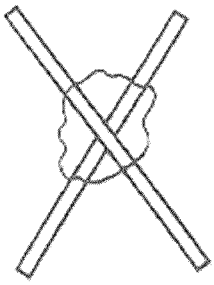


2 structures (2F)

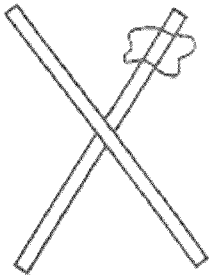
(for the above cases, the nonasbestos particle is < 0.5μm)



1 structure (1F)

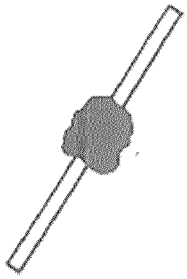


2 structures (2F)

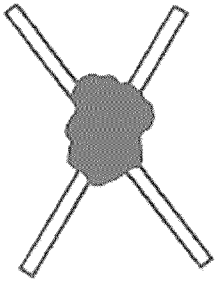


2 structures (2F)

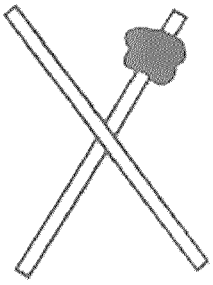
(for the above cases, the nonasbestos particle is ≥ 0.5μm but the ends of the asbestos fibers are visibly not in the nonasbestos particle)



1 structure (1M)



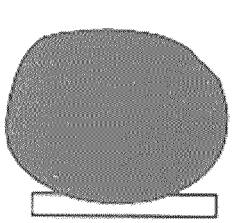
1 structure (1M)



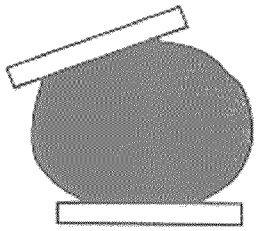
2 structure (1M, 1F)

(for the above cases, the particle is ≥ 0.5 μm and the end of the asbestos fibers may be in the matrix particle)

Figure 6b. Special cases of asbestos associated with nonasbestos.

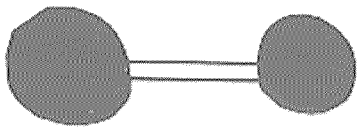


1 structure (1F)



2 structures (2F)

If a fiber or bundle is tangential to a nonasbestos particle so that both ends are free, the fiber is counted as one structure (nonmatrix).

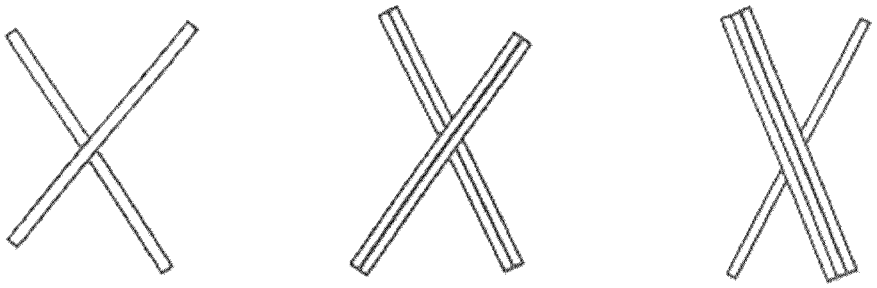


1 structure (1M)

If a fiber or bundle has both ends in nonasbestos particles, the fiber or bundle counts as one structure if the exposed fiber is ≥ 0.5 μm and has a length:width ratio ≥ 5:1.

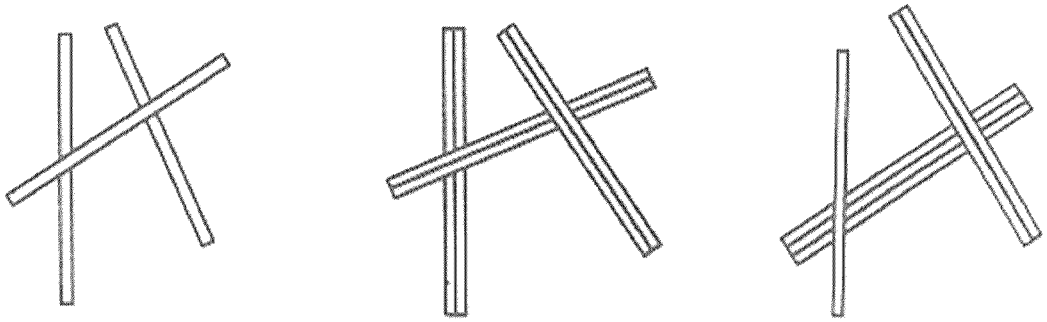
Figure 7. Intersections

Figure 7a. Intersecting fibers, bundles



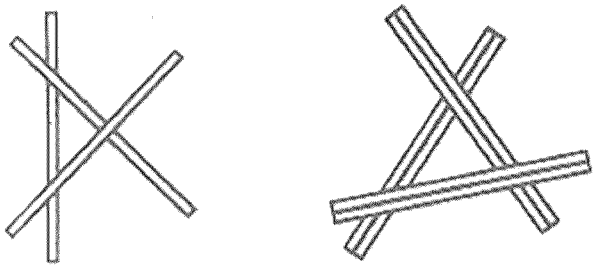
2F 2B 1B, 1F

Count the cases in this row as 2 structures (1 intersection)



3F 3B 1F, 2B

Count the cases in this row as 3 structures (2 intersections)



1C 1C

Count these cases as 1 structure (3 intersections)

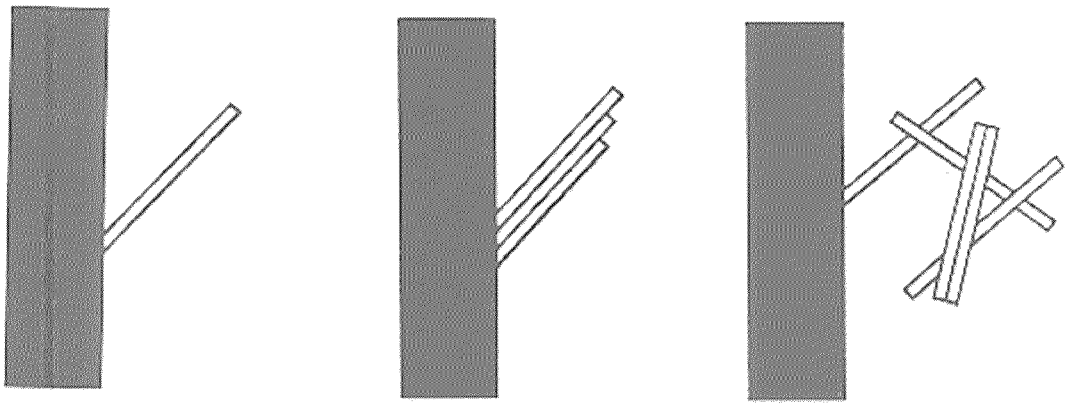
Figure 7b. Special case of intersecting fibers and bundles.

2 structures (1F, 1B)

2 structures (1F, 1B)

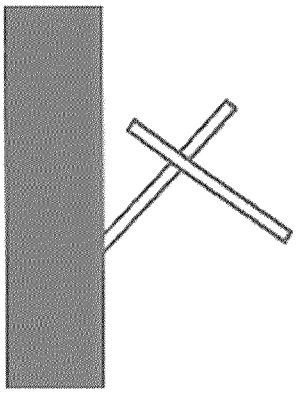
If a fiber or bundle intersects another fiber or bundle but does not protrude by 0.5 µm, it is considered countable if it is ≥ 0.5 µm and has a length to width ratio ≥ 5:1.

Figure 8. Fibers, bundles, clusters on grid bars.

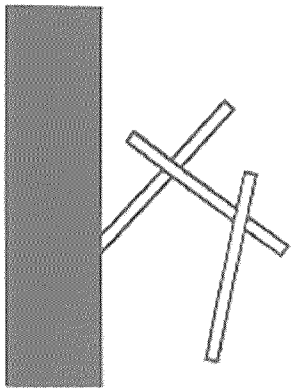


1F 1B 1C

Count the cases in this row as 1 structure



Count as 2 structures (2F)



Count as 3 structures (3F)