NISTIR 5431

Proficiency Tests for the NIST Airborne Asbestos Program - 1990

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Chrysotile

NIST

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U.S. DEPARTMENT OF COMMERCE
Ronald H. Brown, Secretary

TECHNOLOGY ADMINISTRATION
Mary L. Good, Under Secretary for Technology

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
Arati Prabhakar, Director

Preface

The National Voluntary Accreditation Program (NVLAP) at the National Institute of Standards and Technology (NIST) has since 1990 had a program to accredit those laboratories involved in the analysis of airborne asbestos by transmission electron microscopy. As a part of that program, laboratories are sent proficiency tests twice yearly to evaluate their ability to correctly analyze samples and to test the general knowledge of laboratory personnel. The results of the tests are sent to the participating laboratories in the form of a summary report. This NIST Internal Report (NISTIR) contains the instructions and summary reports issued for the proficiency tests in 1990 (PT90-1, PT90-2). Other NISTIRs will be issued for the proficiency tests of subsequent years. The NISTIRs provide a historical record of materials sent to the laboratories for proficiency testing so that they can be referenced in other publications and so that background material can be given to those laboratories entering the accreditation program. The materials can also be used as educational aids. The material in the IRs are copies of the instructions and summary reports sent to the laboratories - if comments are warranted they are given on the chapter title page for the instructions or summary report.

Table of Contents

I. Instructions for PT90-1

This proficiency test consists of two parts: 1) preparation of polycarbonate and mixed-cellulose-ester filters and 2) counting of asbestos structures on grids prepared from filters. For the first part, the laboratories were sent one polycarbonate and one mixed-cellulose-ester filter. Each had zinc-silicate-phospor particles deposited on them as markers. For the counting portion of the test, the laboratories were sent four grids that had been prepared from filters that had chrysotile deposited on them and one grid prepared from a blank filter.

INSTRUCTIONS FOR SAMPLE PREPARATION PROFICIENCY TEST

1. Enclosed in the packet are two 25mm cassettes containing a polycarbonate filter and a mixed cellulose ester filter as indicated by the labels on the cassettes (PC = polycarbonate; MCE = mixed cellulose ester). Tracer particles have been deposited onto the filters to assist in evaluating laboratory sample preparations. The laboratory must prepare and return three grids from each filter for evaluation. These grids are <u>not</u> to be analyzed for asbestos by the laboratory. These guidelines must be followed:

   a. Form 1 must be completed.

   b. 200-mesh finder grids must be used. Finder grids enable the operator to relocate the same area on the surface of the specimen. It is preferred that carbon films grids <u>not</u> be used.

   c. Do not prepare grids from the outer 3mm of the diameter of the filters.

   d. Remaining sections from each filter, measuring at least 3mm X 3mm must be returned to RTI in the containers provided. The sections must not be carbon-coated or altered in any way.

   e. The laboratory may utilize the entire filter for sample preparation except the areas described in c. and d. above.

2. One grid from each filter type will be randomly chosen and evaluated by RTI and/or NIST personnel using the criteria listed in section 4g., Appendix F in the Airborne Asbestos Analysis Handbook. Additionally, the grids will be judged on:

   a. The presence, absence or displacement of tracer particles.

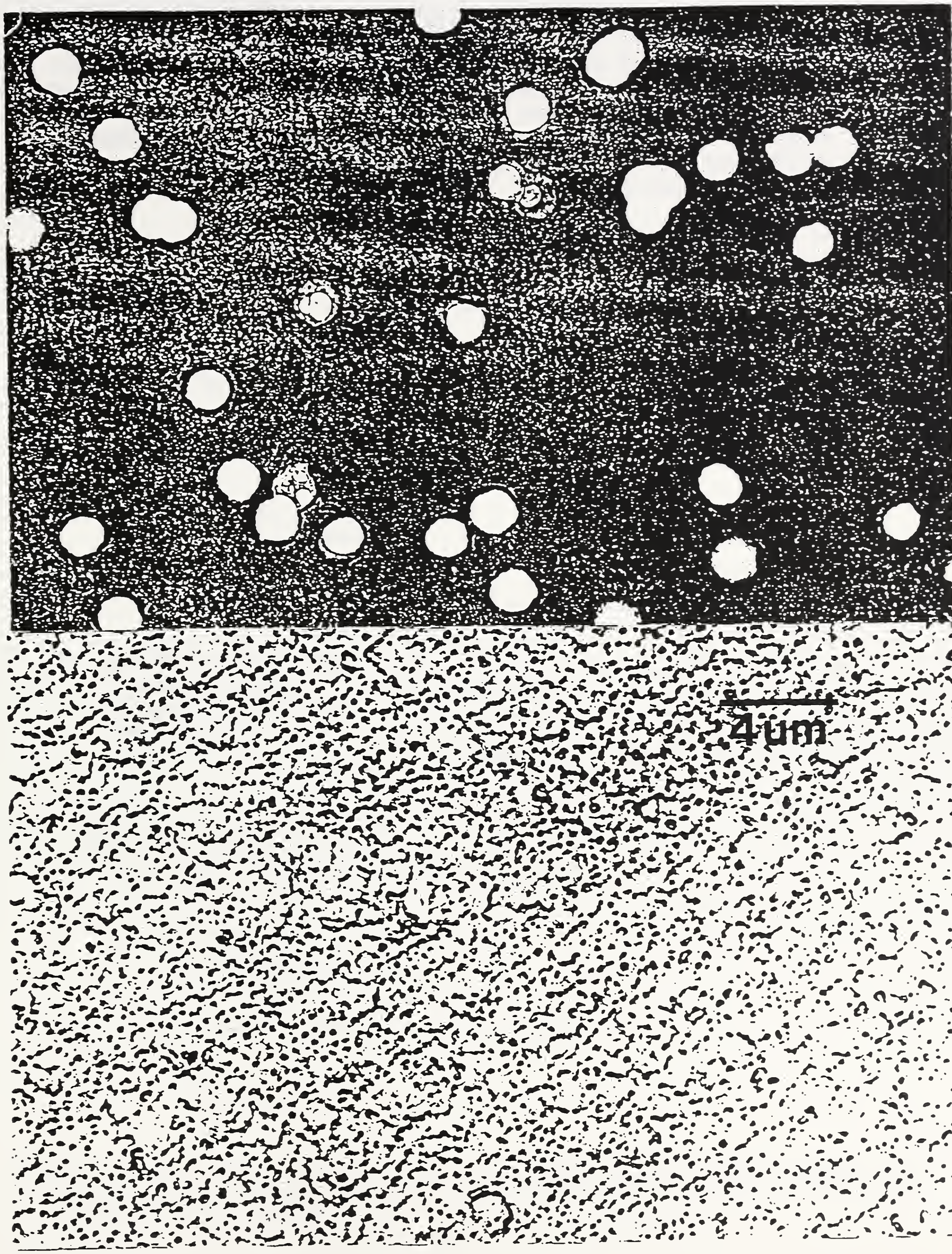
   b. Evidence that steps in the preparation process have been skipped or incorrectly carried out resulting in possible particle loss or in a replica topography or artifacts that could obscure particles or hinder analyses.

The laboratory should evaluate the quality of their grid preparations based on the criteria above and choose the <u>best three grids</u> prepared from each filter. Examples of acceptable polycarbonate and mixed cellulose ester replica preparations are given on the attached sheet.

3. Materials to be returned to RTI:

   a. Form #1.

   b. Prepared grids for evaluation. The grids must be placed in the enclosed grid box (labeled GRIDPREP) using **<u>ROW A slots for POLYCARBONATE</u>** grids and **<u>ROW D slots for MIXED CELLULOSE ESTER</u>** grids.



Examples of acceptable preparations of polycarbonate replicas (top) and mixed cellulose ester preparations (bottom). The holes in the polycarbonate replica correspond to approximately 0.4μm (Note: contrast in these copies of prints is enhanced by the copying process).

INSTRUCTIONS FOR SAMPLE PREPARATION PROFICIENCY TEST (CONT'D)

c. Unused portion of each filter type (as described in 1d. above). An edge of the filter sections must be taped to the plastic containers provided. To ensure that the petri dishes do not open during shipping, please tape the dishes to the piece of the cardboard on which they were sent, placing the piece of tape over the cover of each of the petri dishes.

FORM 1

Laboratory ID #___________

A. General information concerning filter preparation for asbestos analysis: Please fill in the boxes giving approximate values.

	Polycarbonate	Mixed Cellulose Ester
1. Percentage of filters prepped by laboratory over past 2 years		
2. Length of time the laboratory has prepped the filter type (yrs)		
3. Length of time the preparer[1] has prepped the filter type (yrs)		

B. Carbon coating of filters

1. What type of vacuum evaporator was used?

____ carbon rod evaporator

____ carbon thread coater

2. Was the sample rotated during coating?

____ Yes

____ No

[1]The preparer refers to the person who actually prepared the filters for proficiency testing.

C. Preparation of the POLYCARBONATE filter:

The following questions refer to the three grids from the polycarbonate filter that are being returned for evaluation. If answer to the question is not known, write "unknown" (in "other" section if available)

1. Which describes the equipment used in the dissolution of the polycarbonate filter sections?

   ____ a. Jaffe wick only

   ____ b. condensation washer only

   ____ c. Jaffe wick first, then condensation washer

   ____ d. condensation washer first, then Jaffe wick

   ____ e. other, describe:

2. What reagent(s) was(were) used to dissolve the polycarbonate filter?

   ____ a. chloroform

   ____ b. 1-methyl-2-pyrrolidone

   ____ c. other, describe:

3. What was the temperature of the room in which the filters were dissolved?

   ____

4. If the Jaffe wick was used:

   a. How long were the grids exposed to chloroform? __________

   b. The filter sections were placed on the grid

      ____ carbon side up

      ____ carbon side down

   c. Which describes the physical setup of the Jaffe wick?

      ____ filter paper on a saturated sponge

      ____ lens paper on a metal mesh

      ____ other, describe:

5. If the condensation washer was used:

   a. How long were the grids washed? ____

   b. The filter sections were placed on the grid

      ____ carbon side up

      ____ carbon side down

6. Were any nonstandard methods used to prepare the grids?
(If so, describe the methods used.)

D. Preparation of the MIXED CELLULOSE ESTER filter:

The following questions refer to the three grids from the mixed cellulose ester filter that are being returned for evaluation.

1. Which item describes the technique used to collapse the filter?

____ a. acetone hot vapor

____ b. acetone vapor (not heated)

____ c. acetone liquid

____ d. dimethylformamide (DMF)

____ e. 50% DMF, 35% distilled water, 15% glacial acetic acid

____ f. 50% DMF, 50% distilled water

____ g. acetonitrile

____ h. other, describe:

2. To dry the filter section(s) after collapse, what process was followed?

____ a. heating the slide and filter section on a hot plate

temperature ________ length of time ________

____ b. heating the slide and filter section in an oven

temperature ________ length of time ________

____ c. no action taken

____ d. other, describe:

3. To etch the filter section(s), what process was followed?

____ a. etching in a plasma etcher using air

____ b. etching in a plasma etcher using oxygen

____ c. the section(s) were not etched

____ d. other, describe:

4. Which describes the equipment used in the dissolution of the mixed cellulose ester filter sections?

____ a. Jaffe wick only

____ b. condensation washer only

(Next page Question 4 Con't)

____ c. Jaffe wick first, then condensation washer

____ d. condensation washer first, then Jaffe wick

____ e. other, describe:

5. What reagent(s) was(were) used to dissolve the mixed cellulose ester filter sections?

____ a. DMF

____ b. acetone

____ c. both, give a brief description (i.e. DMF in Jaffe wick, acetone in condensation washer):

____ d. other, describe:

6. If the Jaffe wick was used:

a. How long were the grids exposed to the reagent? ____

b. The filter sections were placed on the grid

____ carbon side up

____ carbon side down

c. Which describes the physical set up of the Jaffe wick?

____ filter paper on a saturated sponge

____ lens paper on a metal mesh

____ other, describe:

7. If the condensation washer was used:

a. How long were the grids washed? ____

b. The filter sections were placed on the grid

____ carbon side up

____ carbon side down

8. Were any nonstandard methods used? ____
(If so describe the methods used.)

INSTRUCTIONS FOR ASBESTOS STRUCTURE COUNTING PROFICIENCY TEST

1. Fill out Form 2, general information.

2. Enclosed in the packet are grid boxes numbered one through five. Each grid box contains one indexed finder grid. Two grid squares from each of the grids will be counted.

3. There are two acceptable approaches that can be used by the laboratories to perform the analyses:

   a. One operator analysis - the 10 grid squares chosen are counted by one analyst. The grid squares are not split between more than one analyst. The counts of this single analyst are reported for evaluation of structure counting proficiency of the laboratory. Follow the remaining instructions below except #8.

   b. Verified analyses - the set of 10 grid squares chosen are counted by more than one analyst. Each analyst counts the same set of 10 grid squares. The counts are compared, true positives determined, and a combined count is reported for evaluation. Follow the remaining instructions below including #8.

In either case, each analyst that participates must count the full set of ten grid squares.

4. Each of the five grids examined must be oriented as described in Form 3 instructions so verified counts may be performed at a later date, if necessary.

- Read the instructions for filling out Form 3
- Correctly orient one of the grids from the grid boxes in the TEM specimen holder
- Fill out Form 3

5. Examine the grid squares for suitability using the following order (labelling scheme is described in instructions for Form 3):

F1, E1, H1, F5, E5, H5, F10, E10, H2, F6, E6, H6, F15, H3

Choose for counting the first two grid squares that are nonadjacent and that have the following characteristics:

- Less than 5% of the area has holes or tears
- Less than 5% of the area is dark from incomplete dissolution
- The replica is not doubled or folded
- The area is uniformly loaded

If none of the grid squares in the list are acceptable, other grid squares may be randomly chosen from the grid.

INSTRUCTIONS FOR ASBESTOS STRUCTURE COUNTING PROFICIENCY TEST (CONT'D)

6. Read the instructions for filling out Form 4a. Count the asbestos structures on two grid squares from each of the five grids. Use single analyst or verified count technique. Fill out Form(s) 4a during the count using as many copies of the form as necessary to complete the count. There should be Form(s) 4a for each analyst counting the grid square and for each grid square.

7. Fill out Form 4b at the completion of each grid square count. There should be one Form 4b for each grid square analyzed and for each analyst. Staple Forms 4a to Forms 4b.

8. **For Verified Analysis only**: Fill out Forms 5a for each grid square analyzed. More than one copy of Form 5a may be necessary to include sketches of all the asbestos structures counted. To fill out Forms 5a, all analysts examining the grid square should collectively compare their sketches from Forms 4a. All identical sketches from the grid square in question should be sketched onto Form 5a. Some discrepancies may occur, such as one analyst finding an asbestos structure that the others do not, or vice versa, misidentification of structures, measurement differences that make the structure uncountable, and so on. Discrepancies should be resolved by re-examination of the grid square by all analysts. Orient the grid in the specimen holder as previously indicated on Form 3 for the re-examination. Using the same scan direction, relocate the area of the grid in question and "verify" the questionable structure.

9. Complete Form 6. Record the grid squares counted from each of the grids, the analyst initials, and only one value for the structures counted in each of the grid squares. At the bottom of Form 6, indicate if the recorded countable asbestos structure values were obtained through single operator analysis or by verified count analysis.

10. Fill out and review the checklist provided to ensure that all materials are returned to RTI.

11. Laboratories are requested to return a hard copy of an asbestos EDXA spectra and a print or negative of an SAED pattern from an asbestos structure identified in this proficiency test.

FORM 2

General Information

LABORATORY ID#________________

1. What types of computers are available in the laboratory? (Short programs may be distributed in the future).

____ IBM XT or clone

____ IBM AT or clone

____ IBM 386 or clone

____ Mac II

____ MacSE

____ Other (List) ____

What types of media can the laboratory accept?

____ 3 1/2" 720 kilobyte disk

____ 3 1/2" 1.44 megabyte disk

____ 5 1/4" 1.2 megabyte disk

____ 5 1/4" 360 kilobyte disk

____ Other (List) ____

2. For the structure counting portion of the proficiency test:

What type (Make, Model) of TEM was used for analysis?____________________

____

What type (Make, Model) of EDS system was used?________________________

____

3. What is the calibrated screen magnification of the TEM at the magnification setting used for structure counting?____________________

How was the screen magnification determined?____________________

____

____

4. For each analyst involved in the structure counting portion of the proficiency test, list the approximate length of time (in years) that they have been counting asbestos by TEM?

________yrs. Operator 1, initials __________

________yrs. Operator 2, Initials __________

________yrs. Operator 3, initials __________

INSTRUCTIONS FOR FILLING OUT FORM 3

The following procedure is designed to ensure all laboratories count their grid squares in the same orientation and scan direction.

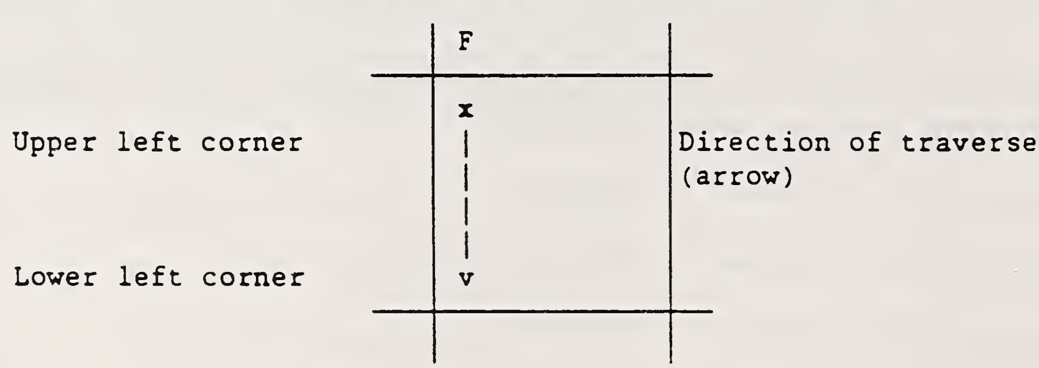
1. Grids sent for structure counting are finder grids. These grids are indexed to make verified counting possible, i.e., you can come back to the same location on the grid for subsequent analyses. Figure 1. shows how the laboratory should record the grid squares which are analyzed.

2. Place a grid into the TEM specimen holder as per laboratory SOP. Insert the specimen holder into the TEM and illuminate the specimen. Find a particle on the grid replica. Increase the magnification to that which is typically used for asbestos analysis by your lab, keeping the particle in the field of view. Make sure the fluorescent screen is lowered. Move the particle using only one stage translation knob. Note the direction in which the particle moves. Record the particle's direction of movement (with a line) on Form 3. Move the particle again, using the other stage translation knob, and sketch that direction of movement relative to the first. This should roughly form a cross, which may not be square with the edges of the paper. The cross represents the translation directions of your microscope at the magnification used for asbestos analysis. Draw a letter "F" onto the cross so the sides of the letter are parallel to the translation directions. and the letter is upright and is not inverted. See example on Form 3.

3. Decrease magnification and locate the letter "F" on the finder grid. Increase the magnification of the TEM to that typically used for asbestos analysis by your lab, keeping the letter F in the field of view. Compare the orientation of the "F" to the cross drawn in step 2. If the letter F is not oriented as shown in your sketch, remove the specimen holder and rotate or invert the grid as necessary to correctly align the grid. This may require several iterations to correctly align the grid.

4. When the correct orientation is found, record the grid's position in the specimen holder as shown in the example of the second part of Form 3. Indicate in your drawing where the straight side and the notched portion of the grid are located. Indicate whether the grid is shiny side up or dull side up in the holder (in most cases, the replica is on the shiny side of the grid). All grids analyzed in this proficiency test should be oriented in the same manner (always check that the letter F is in the correct orientation and that the X-Y translation directions allow translation roughly parallel to the grid bars).

5. Starting point of the traverse for structure counting must correspond to the upper left corner on the grid square. The "X" marks the starting corner of the traverse (Your grid square may be at an angle to that shown in the example):



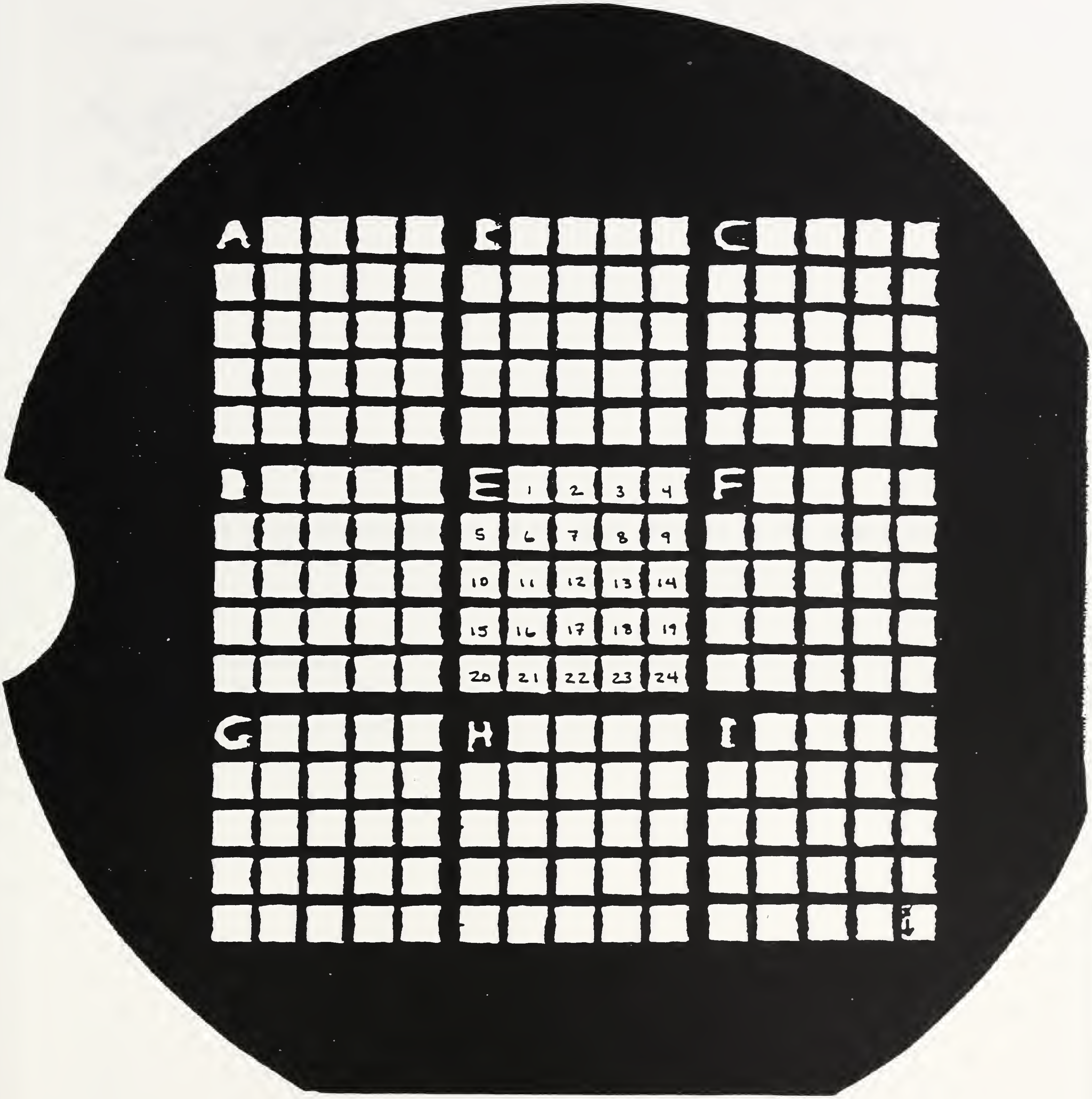


Figure 1. Indexed Electron Microscope Grid. Grids in block "E" show how the grid squares are numbered. Grid square I-24 shows initial start point and scan direction for structure counting.

INSTRUCTIONS FOR FILLING OUT FORM 3 (CONT'D)

The initial direction of traverse must be from upper left corner to the lower left corner of the grid square.

6. Correct grid orientation can be checked by scanning a grid square from the upper left to lower left corner. If correctly oriented, the edge of the grid bar will remain in the field of view during the entire scan. Some allowance must be made for curvature or irregularly shaped grid bars. If the grid is not oriented properly, repeat steps 2 through 4 above.

FORM 3

Grid Orientation

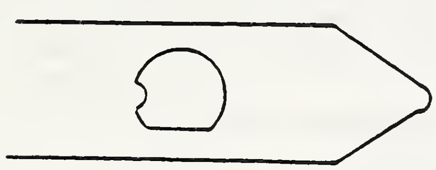
LABORATORY ID#________________

1. Sketch the orientation of the X-Y translation directions of the electron microscope as projected onto the electron microscope stage. Record the letter "F" as shown in the example below:

EXAMPLE:

2. Sketch below the orientation of the grid relative to the sample holder as shown in the example below:

EXAMPLE:



Grid is oriented:

____ shiny side up

____ shiny side down

INSTRUCTIONS FOR FILLING OUT FORM 4a

Structure counting should be done so as to minimize damage to fibers and structures. All fibers or structures containing fibers with an aspect ratio $\geq$ 5:1 and a length $\geq$ 0.5 μm should be recorded. All data should be obtained at 0° tilt of the electron microscope stage. The information should be recorded on Form 4a as follows:

REFER TO THE ATTACHED EXAMPLE OF FORM 4a

1. Record the number of the grid box for the grid being analyzed, the initials of the analyst, date, laboratory ID number, and the page. Select a grid square suitable for counting and record the grid square location. As many copies of Form 4a as necessary may be used. Use separate forms if additional analysts will count the grid square.

2. Record size measurement in micrometers (microns). The dimensions to be recorded are for structures identified as one countable unit. For instance, if a bundle is identified as a countable structure, the dimensions of the bundle are to be recorded (not the dimensions of an individual fiber within the bundle). If a cluster is identified as one countable structure, the dimensions of the cluster are to be recorded. More complicated cases are discussed below.

3. Sketch structures in a fairly detailed manner so they can be relocated or distinguished from other structures. If the structure intersects a grid bar, sketch the orientation of the grid bar relative to the structure. Sketch any other prominent features in the field of view along with the fibers(s) of interest. Be sure to draw the picture with the screen down and to draw the structure in its correct orientation. Also, make sure that the electron microscope stage is at 0° tilt.

4. If there are complicated arrangements of countable structures (corresponding to two or more countable structures):

- Sketch the structures in one box of the form
- Label the fibers, bundles, clusters or matrices (i.e., F1, F2, F3 for fibers one, two and three, or B1, B2, B3, for bundles one, two and three, etc.) SEE EXAMPLE OF FORM 4a ATTACHED
- Define structure dimensions and other information requested on subsequent lines of the form after putting the label in the sketch box.

Follow this procedure only if there are two or more countable asbestos structures in an arrangement. If the number of intersections is greater than three and therefore leads to a designation as a cluster (1 structure) do not label fibers or bundles and do not record information on subsequent lines of the form. For ambiguous structures which do not fit existing categories, photograph the structure and return the prints or negatives with the package to RTI. Note on the print or negative, the grid square from which the micrograph was taken.

INSTRUCTIONS FOR FILLING OUT FORM 4a (CONT'D)

5. Record the presence or absence of a hollow tube as follows:

   0 - No hollow tube
   1 - Hollow tube present

6. For each potentially countable structure obtain information from at least one analytical technique (either SAED or EDXA).

7. For the SAED (Selected Area Electron Diffraction) block, fill out the following codes:

   N - No diffraction pattern observed
   C1 - Diffraction pattern "appears" to be chrysotile
   C2 - Some aspect of the diffraction pattern has been measured and is consistent with chrysotile
   A1 - Diffraction pattern "appears" to be amphibole
   A2 - Some aspect of the diffraction pattern has been measured and is consistent with amphibole
   Ot - "Other"; the diffraction pattern does not correspond to asbestos mineral
   --- - No attempt was made to obtain a diffraction pattern

8. For the EDXA (Energy Dispersive X-ray Analysis) block, fill out the following codes:

   N - No elements observed in spectra
   Element symbols - If elements observed in spectra
   --- - If no attempt was made to obtain a spectra

9. Number of countable asbestos structures block. Record 0 structures if the structures are not asbestos or if the structures do not meet criteria defined in AHERA for countable asbestos structures. Record the countable number of asbestos structures using the AHERA counting rules and modifications included in this package.

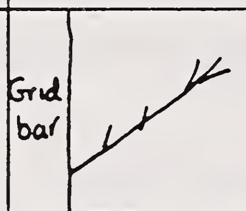
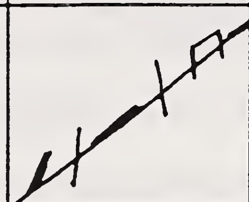
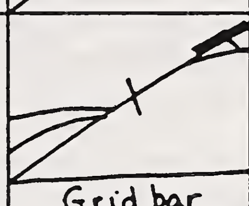
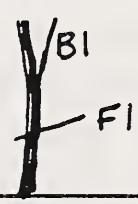
10. F-B-M-C block. Designate asbestos structures as one of the following:

   F - Fiber
   B - Bundle
   M - Matrix
   C - Cluster

11. Identification block. If the structure is asbestos, write the asbestos type. If the structure is not asbestos, write "Other", or give a tentative identification.

Form 4a: Sheet for recording structure counts of one grid square by one analyst.

Laboratory # Example
Grid Square: C9
Analyst (init): A.B.C.
Grid Box: 1
Pg: 1
Date: 1/24/90

Length (μm)	Width (μm)	Orientation (sketch)	Hollow Tube	SAED C1,C2,A1 A2 OT(-) N	EDXA List Elements	#Countable Structure	F-B C-M	Identification
11.2	.5		1	C1	—	1	C	Chrysotile
.5	.1		O	N	Mg, Si	1	F	Chrysotile
5.6	.1		1	C1	Mg, Si	1	C	Chrysotile
10	.5		1	C1	Mg, Si	1	C	Chrysotile
								
4.7	.2	B1	1	C1	—	1	B	chrysotile
.7	.1	F1	1	C1	—	1	F	chrysotile
2	.3		1	C1	—	1	B	chrysotile

Form 4a: Sheet for recording structure counts of one grid square by one analyst.

Laboratory # ________ Analyst (init): ________ Pg: ________

Grid Square: ________ Grid Box: ________ Date: ________

Length (μm)	Width (μm)	Orientation (sketch)	Hollow Tube	SAED C1,C2,A1 A2 OT(-) N	EDXA List Elements	#Countable Structure	F-B C-M	Identification

FORM 4B

Summary Sheet For Grid Square Counts

LABORATORY ID #__________ GRID BOX #__________

GRID SQUARE_______________ ANALYST(INIT)_______

INSTRUCTIONS:

1. Fill out one Form 4b for each grid square analyzed. If more than one operator analyzes the grid square, fill out a separate Form 4b for each analyst.

2. Record the grid box #, the grid square analyzed, the analyst's initials and laboratory ID#.

3. Fill out the requested information in questions 1, 2, and 4.

4. Write the total number of countable asbestos structures from Form(s) 4a in #3 below.

5. Staple Form 4b behind Form(s) 4a. THERE SHOULD BE ONE SET OF FORMS 4a AND 4b FOR EACH GRID SQUARE AND FOR EACH ANALYST ANALYZING THAT GRID SQUARE.

---

1. BEGINNING TIME FOR COUNT ____________

   END TIME FOR COUNT ____________

   TOTAL COUNT TIME ____________

2. Indicate any problem features on the grid replica of the square counted which may have interfered with the count:

   ____ HOLES OR TEARS IN REPLICA

   ____ SPLITTING OF REPLICA

   ____ CLOUDED AREAS

   ____ OTHER, DESCRIBE:

3. How many countable asbestos structures were identified in the grid square (total the number of countable asbestos structures from Form 4a)?

____

4. How many analysts counted this grid square? _____

Form 5a: Sheet for recording structures identified in a verified count of one grid square. Instructions for recording information for each structure (length, width, etc....) are located in Instructions for Filling our Form 4a, p.15.

Laboratory # ________ Analyst (init): ________ Pg: ________
Grid Box: ________ Grid Square: ________ Date: ________

Length (μm)	Width (μm)	Orientation (sketch)	Hollow Tube	SAED C1,C2,A1 A2 OT(-) N	EDXA List Elements	#Countable Structure	F-B C-M	Identification

FORM 5B

Summary Sheet for Verified Count Analysis

LABORATORY ID#________________ GRID BOX #__________

GRID SQUARE___________________ ANALYSTS (INIT) __________

____

____

INSTRUCTIONS:

1. Use this form only if Verified Analysis was performed.

2. Total and record the number of verified asbestos structures identified in this grid square.

Total number of verified asbestos structures on this grid square__________

Form 6. Grid square counts to be used for evaluation of laboratory's proficiency in structure counting (Instructions for completing Form 6 are located in #9 of Instructions for Asbestos Structure Counting Proficiency Test, page 9.)

Laboratory ID #__________

RECORD ONLY ONE VALUE IN # COUNTABLE ASBESTOS STRUCTURES BLOCK

	Grid Square	Analyst(s) Initials	#Countable Asbestos Structure
Grid box #1			
Grid box #2			
Grid box #3			
Grid box #4			
Grid box #5			

Check the method used to count the grid squares:

_____ Single operator analysis

_____ Verified count analyses

Explanation of Modifications to AHERA Counting Rules For NIST Proficiency Testing

The following is a list of NIST clarifications and amendments to the asbestos structure counting rules of the EPA Interim Transmission Electron Microscopy Analytical Methods--Mandatory and Nonmandatory to be used in the NIST proficiency testing. The purpose of these rules is not to change or in any way impact on the EPA method but rather to attain reasonable interlaboratory precision for structure counting methods by giving all participating laboratories an equivalent set of counting rules for the NIST proficiency testing program.

Intersecting fibers, bundles:

AHERA rules: The AHERA rules define an intersection as, "nonparallel touching or crossing of fibers...". A bundle is defined as a "structure composed of three or more fibers in a parallel arrangement..."(see Figure 2). A strict interpretation of these rules would imply that if bundles cross then there are multiple intersections ($\geq$ 3 intersections). Therefore the arrangement of crossing bundles would qualify as a cluster and would be counted as one structure. However, from a previous round robin study, it is clear that laboratories have had many differing interpretations for these types of arrangements. The modification for NIST proficiency testing is a common-sense interpretation.

Modification of AHERA rules for NIST proficiency testing: A single intersection is defined as, "nonparallel touching or crossing of fibers (single or double) or bundles" (a double fiber consists of two parallel, touching fibers, and would by itself count as one structure). This modified definition of an intersection means that an arrangement of two intersecting fibers (single or double) or two intersecting bundles count as two structures. (See first page of Figure 3)

Nonstandard bundles:

AHERA rules: There is no ruling for nonstandard bundles.

NIST proficiency testing rule: Nonstandard bundles include those with splayed ends, those that are bent and those that contain fiber(s) that have bowed away from the main bundle. These cases will count as one bundle. Examples are illustrated on second page of Figure 3.

Bundles and clusters containing noncountable asbestos structures:

Note: A noncountable asbestos structure is defined as an asbestos fiber that is <0.5 μm and/or has a length:width ratio <5:1.

AHERA rules: These cases are not dealt with in the AHERA rules.

NIST proficiency testing rule: If the 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 treated as if it were not present. Examples are given on the third page of Figure 3.

Fibers, bundles, clusters on grid bars:

AHERA rules: There is no ruling for fibers, bundles or clusters on grid bars. (See Figure 2)

NIST proficiency testing rule: A fiber (single or double), bundle or cluster touching a grid bar is counted the same way it would be if the grid bar was not there. (See examples on the fourth page of Figure 3)

Closely spaced parallel bundles and fibers:

AHERA rules: The fibers or bundles are counted as separate fibers or bundles if they are separated by more than a fiber diameter. This rule is ambiguous for separated fibers where there are fibers of different diameters. This rule is also ambiguous for separated bundles because it is unclear as to what fiber in the two bundles should be use for the diameter measurement. (See Figure 2)

Modification of AHERA rules for NIST proficiency testing: If a space can be seen between the two parallel fibers or bundles, count them as two separate structures. (See examples on the fourth page of Figure 3)

Asbestos associated with nonasbestos particles - matrices and others:

AHERA rules: A matrix is defined as, "fiber or fibers with one end free and the other embedded in or hidden by a particle. The exposed fiber must meet the fiber definition." The size of the particle is not defined. (See Figure 2)

Modification of AHERA rules for NIST proficiency testing: For proficiency testing, particles that are $<0.5\mu m$ in their longest dimension are considered coatings and do not qualify as matrix particles. Examples of counting of asbestos structures associated with particles are given on the fifth page of Figure 3.

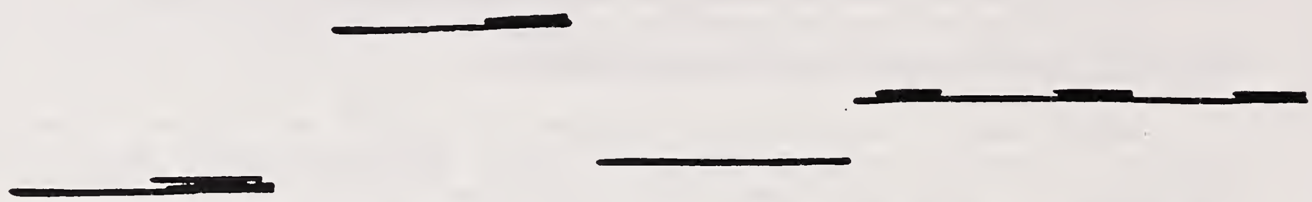
Special case (not sketched): If a countable asbestos fiber or bundle has both ends in matrix particles, the fiber or bundle counts as one structure.

Counting bundles and clusters:

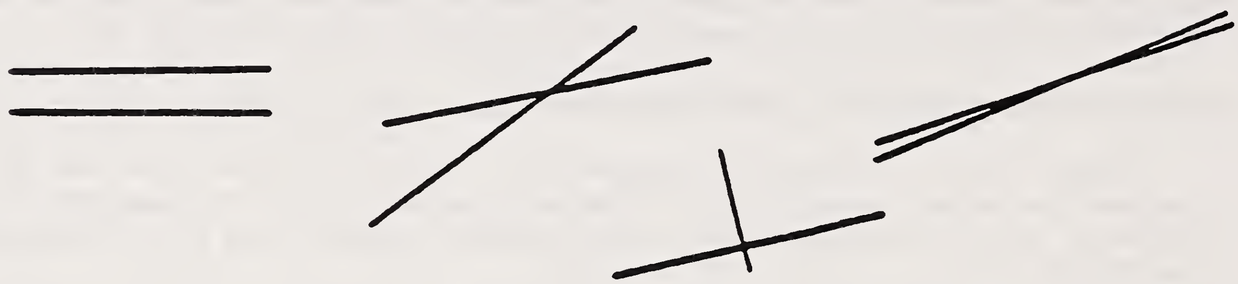
AHERA rules: A fiber is defined as, "greater than or equal to 0.5 μm in length with an aspect ratio (length to width) of 5:1 or greater and having substantially parallel sides". A bundle consists of, "three or more fibers" and a cluster consists of a random arrangement of fibers (see Figure 2). A strict interpretation of these definitions means that a bundle or cluster must contain fibers longer than 0.5 μm and with an aspect ratio of 5:1 or greater. The dimensions and aspect ratios apply to the fibers and not the bundle or cluster itself. This definition will be followed in the NIST proficiency testing. In a previous round robin this ruling was misinterpreted by several laboratories. Examples of correct interpretations are given on the sixth page of Figure 3.

Figure 2. AHERA counting guidelines used in determining number of countable asbestos structures

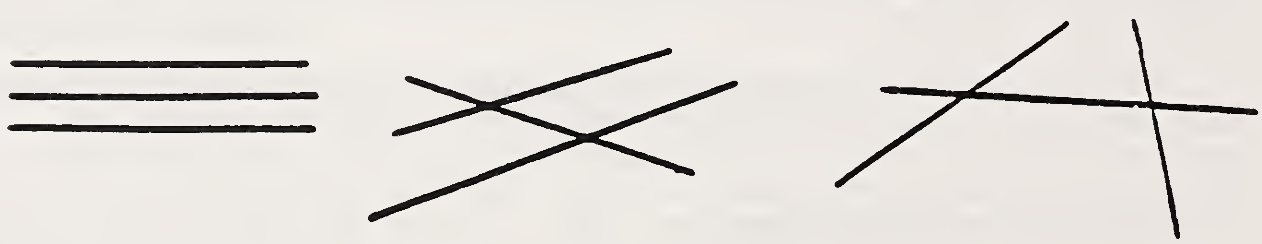
Count as 1 fiber; 1 Structure; no intersections.



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.



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.



Count bundles as 1 structure; 3 or more parallel fibrils less than 1 fiber diameter separation.

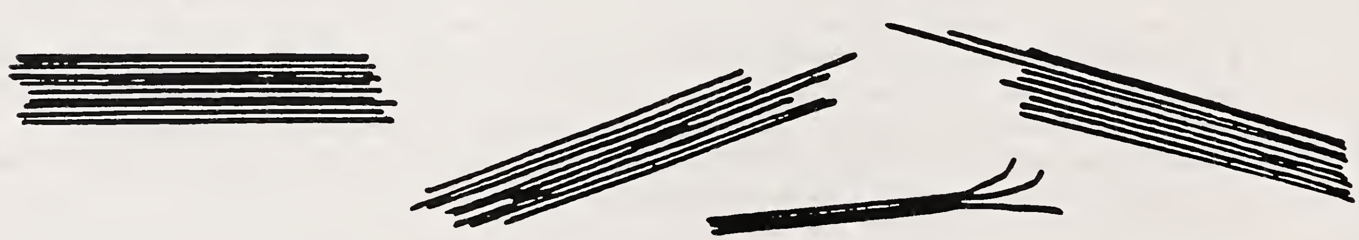
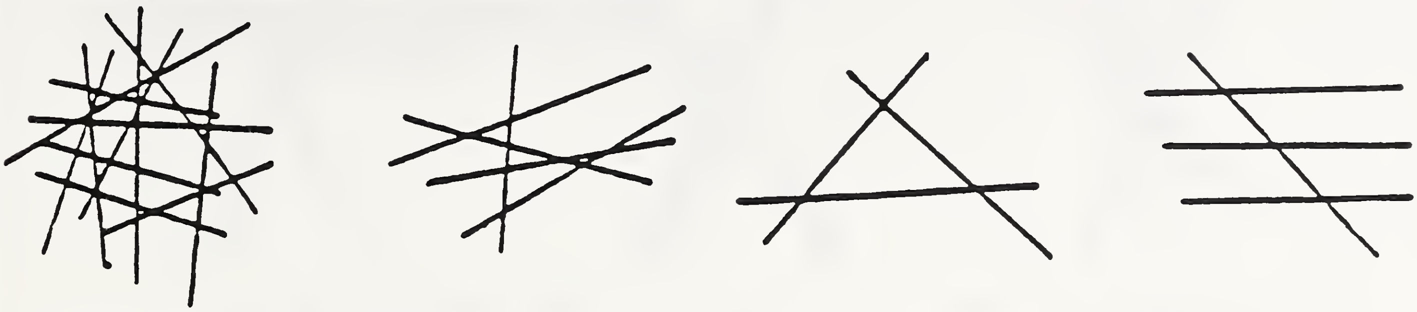
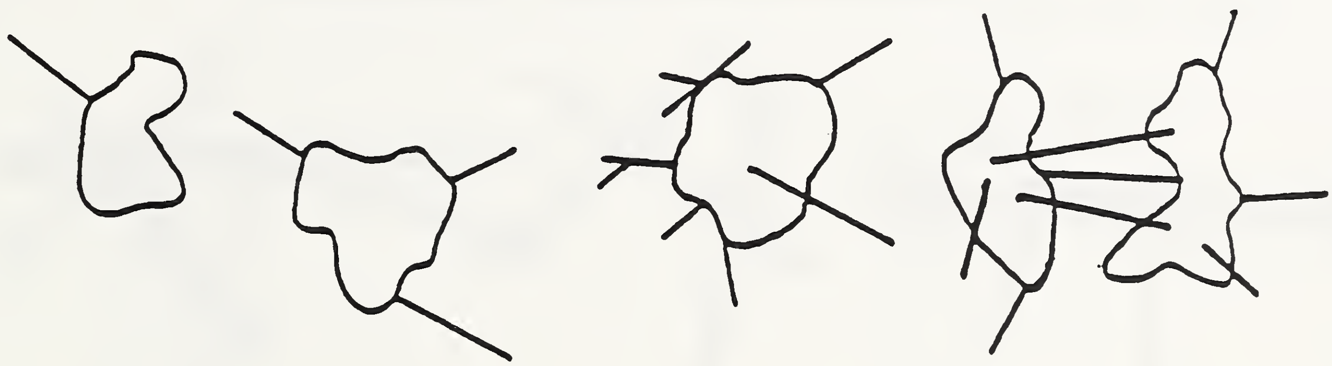


Figure 2. continued

Count clusters as 1 structure; fibers having greater than or equal to 3 intersections.



Count matrix as 1 structure.



DO NOT COUNT AS STRUCTURES:

Fiber protrusion
<5:1 Aspect Ratio

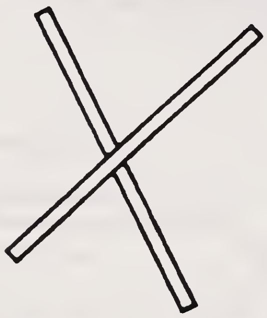
No fiber protusion

Fiber protrusion
<0.5 micrometer

<0.5 micrometer in length
<5:1 Aspect Ratio

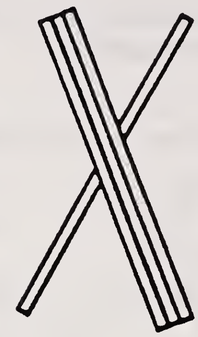
Figure 3. Clarifications and modifications to AHERA counting rules to be used for TEM proficiency testing

Intersecting fibers, bundles:



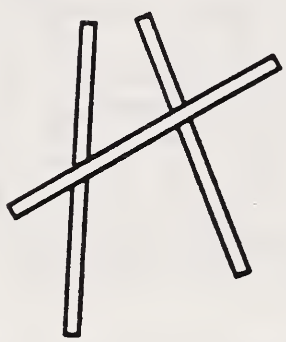
2F

2F

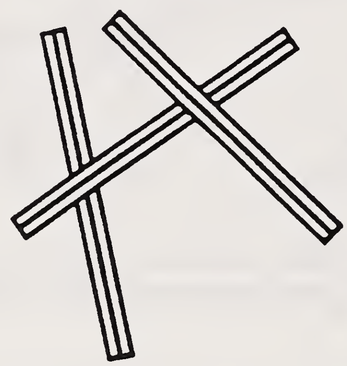


1B, 1F

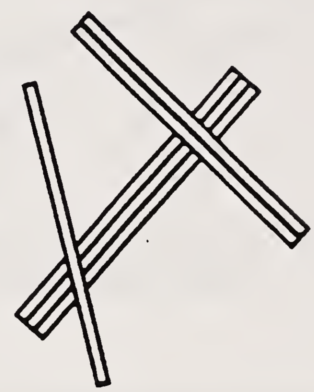
Count the cases in this row as 2 structures (1 intersection)



3F



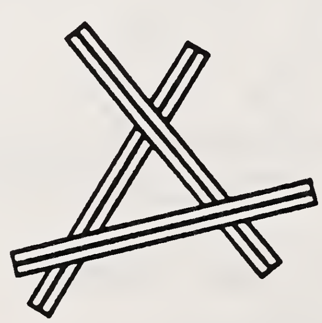
3F



1B, 2F

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

3F



3F

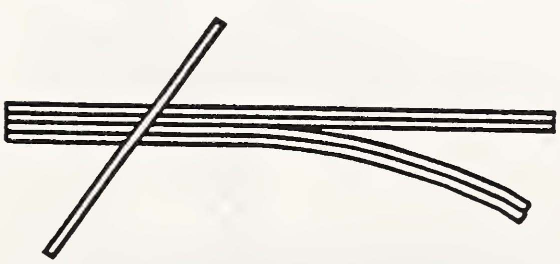
Count these cases as 1 structure (3 intersections)

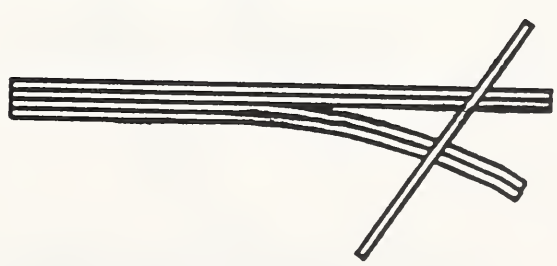
Nonstandard bundles:

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)

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)

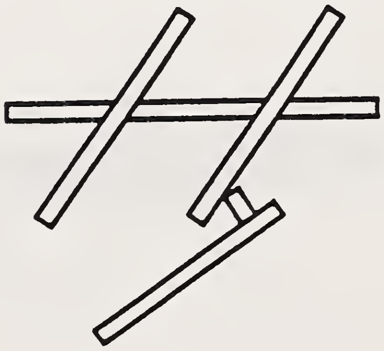
2F

1F

Count as 2 structures

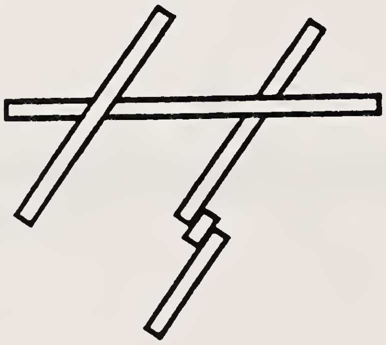
Count as 1 structure

(noncountable asbestos touching but not parallel to countable asbestos)



4F

Count as 4 structures



1B, 2F

Count as 3 structures

Fibers, bundles, clusters on grid bars:

1F

1B

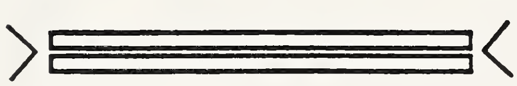
1C

Count the cases in this row as 1 structure

Count as 2 structures (2F)

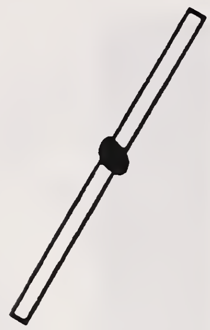
Count as 3 structures (3F)

Closely spaced parallel bundles and fibers:

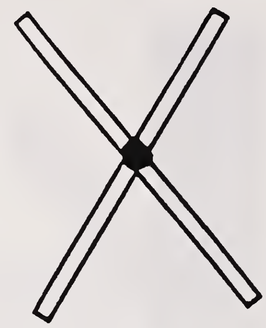


Count the cases on this row as 2 structures (2F)

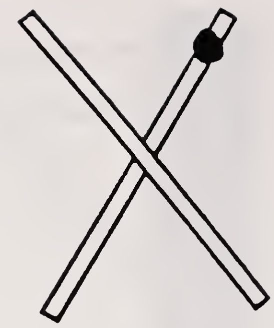
Asbestos associated with nonasbestos particles:



1 structure (1F)

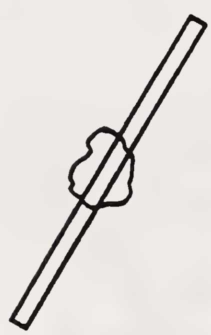


2 structures (2F)

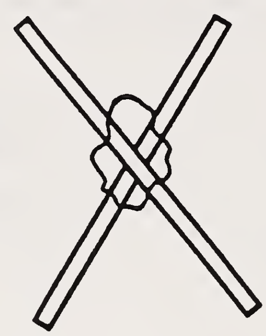


2 structures (2F)

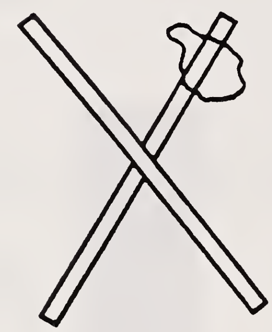
(for the above cases, the nonasbestos particle is < 0.5μm)



1 structure (1F)



2 structures (2F)

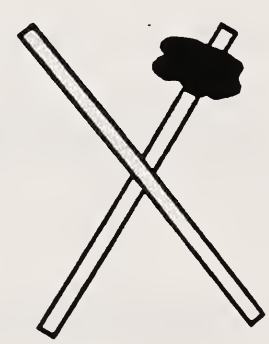


2 structures (2F)

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

1 structure (1M)

1 structure (1M)

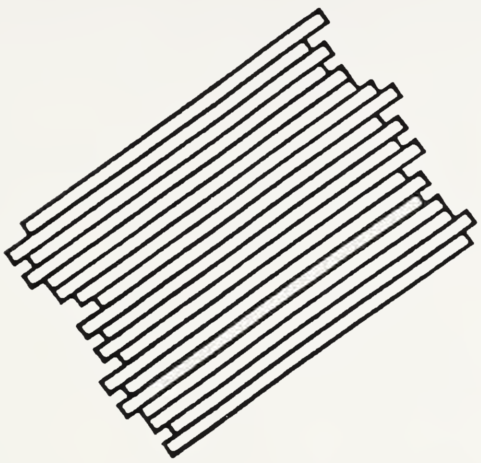


2 structures (1M, 1F)

(for the above cases, the particle is $\geq$ 0.5 μm and the end of the asbestos fibers are possibly in the particle)

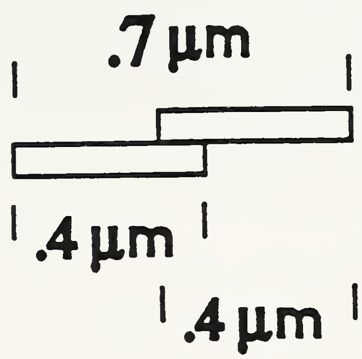
Counting bundles and clusters:

For a bundle or cluster to be countable, a fiber within the structure must have a ratio 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 $\geq$ 0.5 μm,
fibers $\geq$ 5:1 ratio)

count as 0 structures
(cannot distinguish
fiber $\geq$ 0.5 s, $\geq$ 5:1 ratio)



count as 0 structures
(does not have fiber $\geq$ 0.5 μm)

II. Summary Report for PT90-1

ANALYSIS OF SAMPLES FOR THE NIST AIRBORNE PROFICIENCY TESTING PROGRAM, FEBRUARY 1990

The information presented in this report is a summary of the performance of laboratories that participated in the first proficiency test for the airborne asbestos accreditation program. The proficiency test consisted of two major parts - 1) testing the laboratory's ability to prepare carbon replicas of filters used to collect airborne particulate and 2) testing the laboratory's ability to determine the amount of asbestos on a previously prepared replica. Samples returned by the laboratories were evaluated by the National Institute of Standards and Technology (NIST) and/or Research Triangle Institute (RTI) analysts. This summary report contains the overall results of the evaluation of the samples. The individual results of a verified count analysis of one of the grid squares counted by the laboratory is given in Appendix A. If warranted, specific problems that the laboratory should investigate and correct are given in the "Additional comments" section of Appendix A.

PART I - SAMPLE PREPARATION

Samples

To test the capabilities of the laboratories to prepare filters, the labs were sent a cassette containing a 25 mm, polycarbonate (pc) filter and a cassette containing a 25 mm, mixed cellulose ester (mce) filter. Chrysotile and a tracer particle, zinc-silicate phosphor, were deposited onto the filters by the Stanford Research Institute (SRI) using an aerosol generator. The laboratories were instructed to prepare carbon replicas of the filters on finder grids and to return their three best preparations of each filter type to RTI. One-hundred and twelve laboratories returned filter-replica preparations.

Evaluation of returned filter-replica preparations

The carbon replicas prepared by the laboratories were evaluated for problems in several categories. First, the type of grid used by the laboratory to support the replica was determined. The laboratories had been requested to use finder (or indexed) grids. The grids were categorized as 1) nonfinder grids (grids for which analyzed grid squares cannot be uniquely indicated), 2) finder grids with only an assymetric central marking and 3) indexed grids (for which there is an alphabetical and/or numerical marking throughout the grid). As a quality assurance measure, this evaluation of grid type was performed twice (at RTI and NIST), results compared and differences resolved.
The results for evaluation of the grid type are summarized in Table 1:

Table 1. Summary of the types of grids used by laboratories to prepare replicas of filters

Type of grid	Number of laboratories*
Nonfinder	12
Finder (with only a central marking)	26.5
Indexed	73.5

*Some laboratories submitted two different types of grids. In these cases the labs were assigned a value of 0.5 for each grid type.

For the second type of evaluation, one of the three replica preparations submitted by each laboratory for each filter type was evaluated for gross problems in preparation using optical microscope observation. The filter replicas were evaluated for the percentage of the replica that was acceptable - i.e., coherent (containing no holes or splitting) and lacking any obvious sample preparation problems such as uncleared filter. The areal percentage was determined by counting the number of openings covered by replica and counting the number of openings covered by acceptable replica. As a quality assurance measure, approximately 10% of the grids were reanalyzed by a second analyst. The average difference between these repeated analyses was 9% and the pooled standard deviation was 10%. Possible causes of the differences in evaluations include differences in detecting the presence of small splits and holes, differences in the judgement of clearing problems and differences in interpretation of the protocol for evaluation.

The results of the optical microscopy evaluation of the percentage of the replica that is acceptable is summarized in Table 2:

Table 2. Summary of the quality of filter-replica preparations as evaluated by optical microscopy

Percentage of replica that is acceptable*	Number of laboratories	
	pc	mce
< 30%	21	21
30% - 70%	47	41
> 70%	44	50

* It is required by the EPA method and the NVLAP handbook that 50% of the replica be acceptable. The values between 30% and 70% are listed to attain 95% confidence in the values around 50%.

Lastly, approximately one quarter of the grids submitted by the laboratories were evaluated by use of transmission electron microscopy. Replicas were examined for sample preparation problems and artifacts that could potentially affect the number of asbestos structures reported in an analysis

(Turner et al., 1990, Chatfield, 1990). The methods to assess the quality of replica preparations by TEM analysis are still under development. The majority of grids were evaluated to obtain a visual estimate of the area affected by the sample preparation artifact or problem. Concurrently with the visual analysis, a method was developed using image analysis to determine the area affected by a sample preparation problem. This method, though still under development, was used as a quality assurance method to confirm some of the visual estimates.

Of 26 pc preparations examined, nine were considered to be either well-prepared or to have only minor problems. Twenty-seven replicas of mce filters were examined. Twelve of these preparations were judged to be either well-prepared or to have only minor problems. The percentage of labs that were judged to have both their mce and pc preparations as either well-prepared or with minor problems was 22%. The major problems for the pc preparations (in the order of the number of grids affected) include: clouded layer, bubble-like (or overlying), mesh and interconnected pores (Turner et al., 1990). The major problems for the mce preparations include: Type 3 texture (irregularly-shaped features approximately 0.4-1.0 micrometers in size), irregular splits, parallel splits, missing particles, and craters and holes.

Discussion

Approximately 10% of the laboratories used nonfinder grids for their sample preparations. Use of these grids in routine work would hinder subsequent relocation of counted squares and verification of counts obtained by operators. The NVLAP handbook (Steel et al., 1989) requires use of finder grids.

The results of the evaluation of filter-replica preparations by optical microscopy showed that for each filter type, more than 15% of the preparations did not meet the EPA method (Federal Register, 1987) and the NVLAP handbook requirement that preparations used for analysis have at least 50% acceptable replica.

The results of the evaluation of filter-replica preparations by TEM showed that the replicas contained problems that may affect the analysis of asbestos. These results are consistent with the results obtained from two previous round robins. The results of all three interlaboratory studies indicate that improvements in the method of preparation of filter replicas are needed.

PART II - STRUCTURE COUNTING

Samples

To test the capabilities of the laboratories to analyze accurately asbestos-containing carbon replicas, the labs were sent five prepared grids, each in separate grid boxes. Four of the five grids contained filter replicas obtained from a set of mce filters loaded with chrysotile. The remaining grid contained a replica prepared from a blank mce filter.

Chrysotile was deposited by SRI onto a set of 25 mm, mce filters using their fluidized-bed, aerosol generator. After deposition of the chrysotile, the filters were sent to NIST. A plan for sampling the filters was developed with the assistance of statisticians at NIST.

Carbon replicas were prepared from the filters at NIST. Replicas were prepared as follows: 1) filter sections were collapsed with a solution of dimethyl formamide (DMF) and water, 2) collapsed sections were dried on a hot plate, 3) the sections were carbon-coated, and 4) the sections were dissolved away using DMF in a Jaffe wick followed by acetone in a condensation washer. Blank filter sections were prepared with each loaded filter preparation. A filter replica prepared from these blank filter sections was sent to each laboratory as one of the five filter replicas to be analyzed.

The suitability of the replica preparations was checked by NIST and RTI. Each carbon replica was checked on the light microscope for coherence and obvious preparation problems. Approximately 10% of the preparations were checked by use of transmission electron microscopy. Poor filter replicas were rejected.

The filter preparations were packaged by RTI so that each laboratory received five grids, each in a separate grid box. The 113 laboratories participating in the study were asked to analyze two grid squares from each of the five grids. The methods of analysis were specified in a detailed set of instructions that covered grid orientation, initial traverse direction and starting point, counting rules and recording sheets. The grids and the analyses were returned by the laboratories to RTI.

Summary of results reported by laboratories

A total of 226 grid squares were counted by the laboratories from blank filter preparations. The laboratories reported zero structures per grid square for 218 of these grid squares. The remaining eight grid squares contained 21 structures. The distribution of counts are shown in the histogram of Figure 1. The average blank count for the 226 grid squares is 0.09 structures per grid square.

A histogram of the structures reported by the laboratories for the loaded filters is shown in Figure 2. The mean value reported by the laboratories is 14.0 structures per grid square, the standard deviation is 6.3, the minimum value reported is 0 structures and the maximum value is 62 structures. The histogram shows a roughly symmetric distribution with five structure counts greater than thirty-five structures per grid square. At the low end of the distribution, there are an anomalous number of grid squares with zero structures per grid square.

Evaluation of structure counting

The initial intent of this portion of the proficiency testing was to determine the validity of the results reported by the laboratories using statistical methods along with limited verified counting by NIST and RTI. However, the data proved not amenable to statistical methods - some of the structure counts in both the high and low ends of the distribution were verified and they proved valid. Therefore, it was decided that for each laboratory, one grid square of the eight loaded with chrysotile would be verified by an RTI or NIST analyst. The method used is a variation of the verified counting technique reported by Steel and Small (1985). One-hundred and thirteen grid squares were verified by NIST and/or RTI analysts.

Quality assurance and calibration

An extensive quality assurance program was established for the acquisition of data related to verified count analysis of the laboratories' structure counts. The quality assurance program included calibration of instrumentation and TEM operators. As part of the quality assurance program, the laboratory's name was withheld from the NIST/RTI analysts to eliminate any potential prejudice due to knowledge of a laboratory's previous performance.

The magnification of the transmission electron microscopes at NIST and RTI were calibrated using a replica of an optical grating with 2160 lines per mm. The magnifications at the microscope screen and on electron micrographs were calibrated. All fibers and structures that were close to the 0.5 micrometer size limit were recorded on electron micrographs and measurements were made from the negative.

The TEM analysts were calibrated by duplicate verified counts at NIST and/or RTI. Samples were exchanged between the two institutions and analyses compared. Of the 113 analyses of structure counts reported in this study, over 30% were counted by more than one RTI and/or NIST analyst.

To assure that no contamination was introduced during handling of the grids counted by the laboratories, blank carbon film grids were examined after every four to ten verified counts. No asbestos fibers were detected in the blank checks.

Definitions

Several abbreviations are used in the remainder of the report that refer to the categories of structures determined by verified analysis. The abbreviations and their definitions include the following:

SR - structures reported by the laboratory.

TNS - total number of structures - total number of countable structures found on the grid square.

TP - true positive - a structure verified to be a countable asbestos structure.

FP - false positive - a structure incorrectly reported as a countable asbestos structure.

FN - false negative - a countable asbestos structure that was not recorded as a countable structure. The false negative structures were divided into two categories, FNA and FNB, defined below.

FNA - false negative, type A - a countable asbestos structure that was found by a laboratory (drawn on the laboratory's count sheet) but not reported as a countable structure. Commonly the structures were not recorded by the laboratory due to either: 1) confusion with the counting rules or 2) incorrectly finding that a structure is below the 0.5 micrometer size limit.

FNB - false negative, type B - a countable asbestos structure that was not reported by the laboratory and presumably not found by the laboratory.

NL - not located - structures reported by a laboratory that could not be found by the analyst verifying the count.

Results of verification of loaded grid preparations

One grid square from each laboratory was randomly chosen to be verified by NIST and/or RTI analysts. If the TNS value for the grid square count was less than three, a second grid square was randomly chosen for evaluation. The results of the verified analysis for the individual laboratory receiving this report is given in Appendix A. A summary of the values obtained by all of the 113 laboratories is given in Table 3:

Table 3. Summary of results of verified counts expressed in number of structures

	Average Val.	St. Dev.	Minimum	Maximum
TNS	16.7	5.2	3	31
SR	14.9	5.4	1	30
FP	1.4	1.6	0	7
FNA	2.0	1.9	0	12
FNB	1.2	1.8	0	11
NL	0.04	0.23	0	2

The average percentage of TP, FP, FNA and FNB obtained by the laboratories are summarized in Table 4:

Table 4. Summary of results of verified counts expressed as a percentage of TNS

	Average percentage
TP/TNS	81%
FP/TNS	9%
FNA/TNS	12%
FNB/TNS	7%

Histograms of the distribution of TP/TNS, FNA/TNS, FNB/TNS and FP/TNS are given in Figures 3-6.

Grid squares with counts falling outside the values obtained by 95% of the laboratories for FNA/TNS, FNB/TNS or FP/TNS were examined more closely. Results falling outside those attained by 95% of the laboratories correspond to the following criteria:

FNA/TNS - $> 25\%$
FNB/TNS - $\geq 30\%$
FP/TNS - $\geq 35\%$

The counts fitting the criterion above for high FNB/TNS values were evaluated by at least two NIST/RTI analysts to assure the correctness of the count. Laboratories with counts falling in this category are noted in the "Additional comments" section of Appendix A.

The counts fitting the criteria above for high FNA/TNS and FP/TNS values were reviewed to determine the nature of the errors that resulted in the high FP or FNA counts. For this proficiency test, we considered two types of errors to be minor: 1) those errors associated with size measurement near the 0.5 micrometer size limit and 2) those errors resulting from misinterpretation of the modified counting rules given in the instructions for proficiency testing. Errors considered to be major include: 1) errors resulting from misinterpretation of EDS spectra or of electron diffraction patterns or 2) errors associated with misapplication of the basic AHERA rules. If the high FNA/TNS or FP/TNS values were due to major errors, the nature of the error is noted in the "Additional comments" section of Appendix A.

Further notes were placed in the "Additional comments" section of Appendix A for other errors noted in the verified count. Examples of such errors include misrecording of the number of countable asbestos structures on various forms and counting grid squares that have severe sample preparation problems such as folded replica.

Discussion

The results of the structure counting portion of the proficiency test show that the mean value obtained for TP/TNS is greater than 0.80. This is an excellent result considering the complexity of some of the structure arrangements that occurred in the materials used in this proficiency test. The values for FP/TNS and FNA/TNS are largely due to a need for improved definition or simplification of the counting rules. NVLAP will be collecting data on an improved method for structure counting and will issue a new method in the future.

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Steel, E.B., S. Turner and H.W. Berger **1989**, NVLAP Airborne Asbestos Analysis Program Handbook, NISTIR 89-4137.

Turner, S., E.B. Steel and E.S. Landis **1990**, Problems and artifacts on extraction replicas of membrane filters. Specimen Preparation for Transmission Electron Microscopy of Materials, II., R. Anderson, Ed, Materials Research Society Symposium Proceedings, Vol. 199, 157-166.

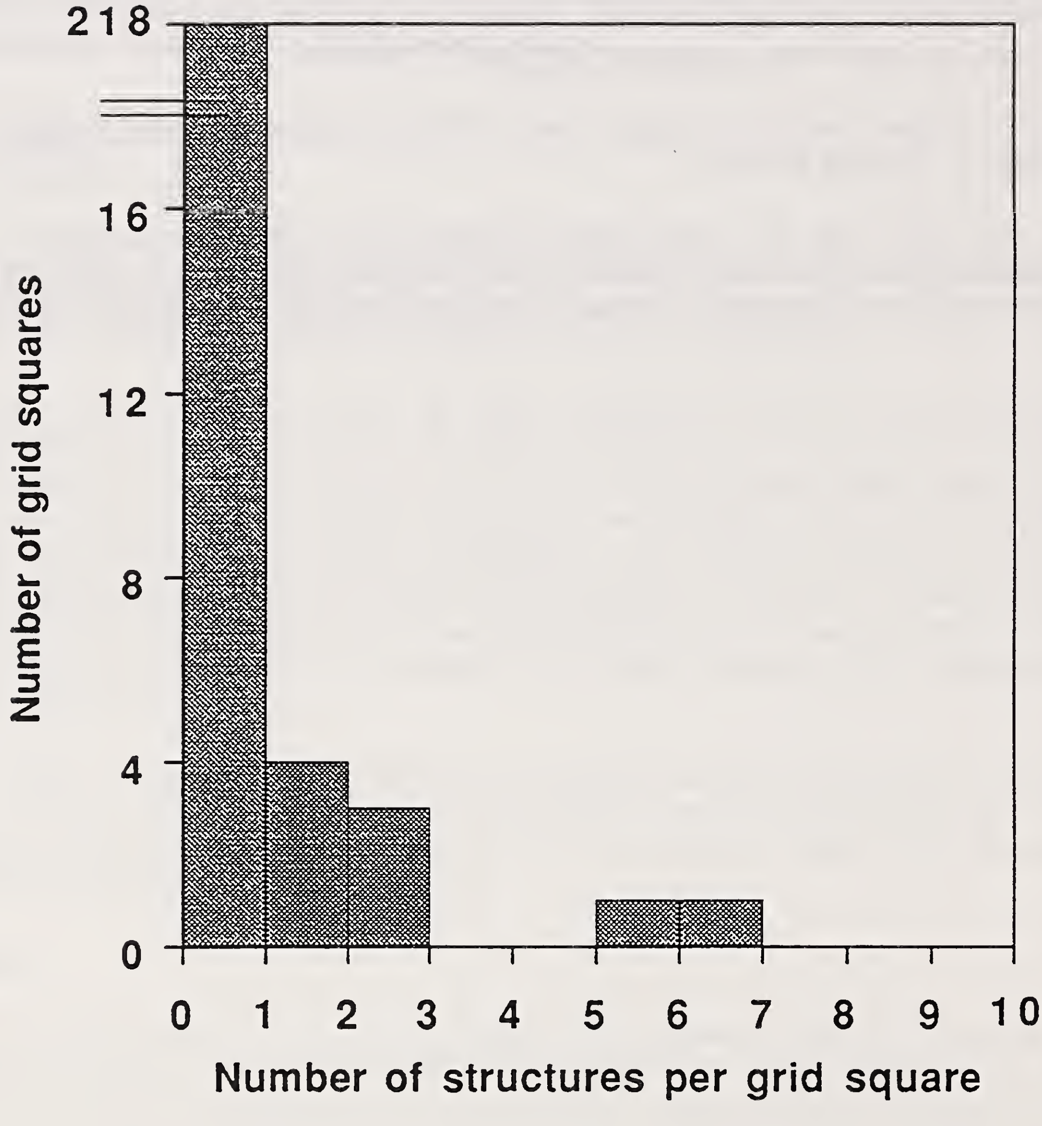


Figure 1. Histogram of the number of structures reported per grid square by the laboratories for grids that were prepared from blank filters.

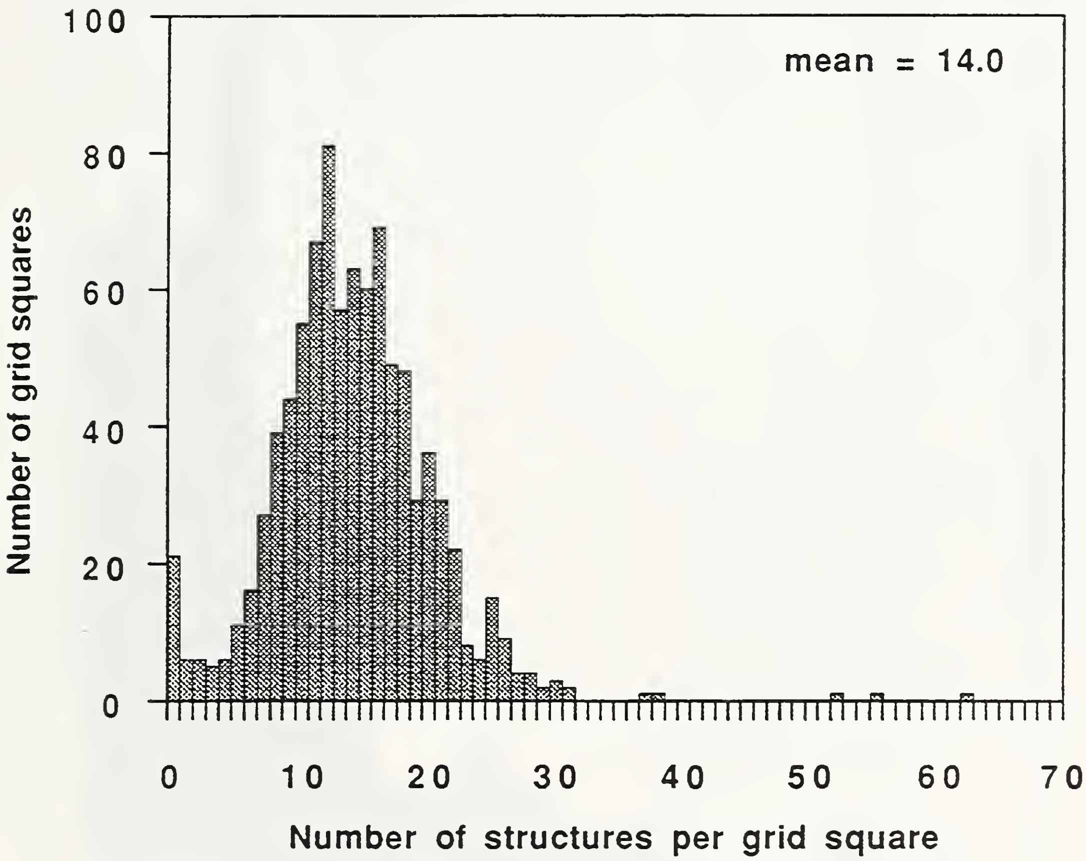


Figure 2. Histogram of the number of structure counts reported per grid square by the laboratories for grids that were prepared from filters containing chrysotile.

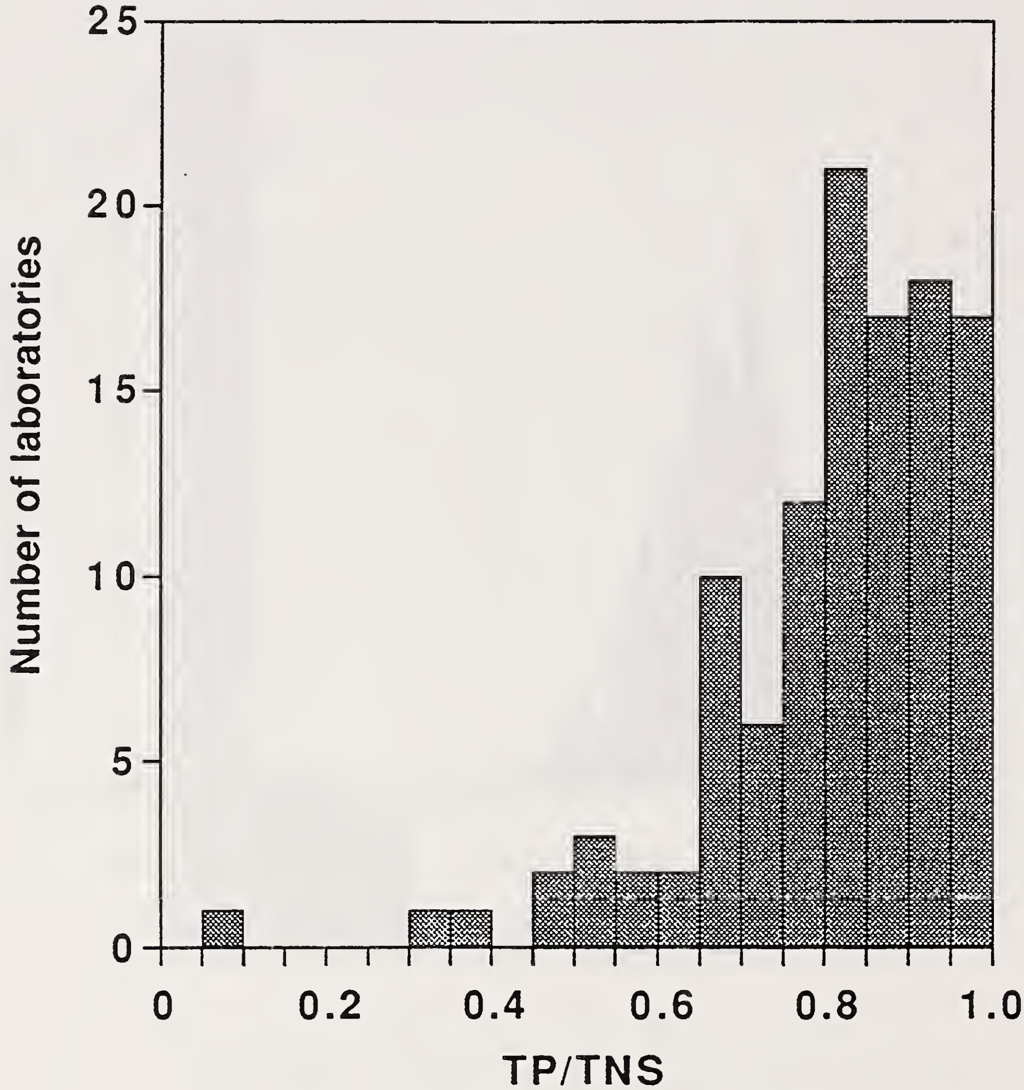


Figure 3. Histogram of the distribution of values obtained for TP(true positives)/TNS(total number of structures).

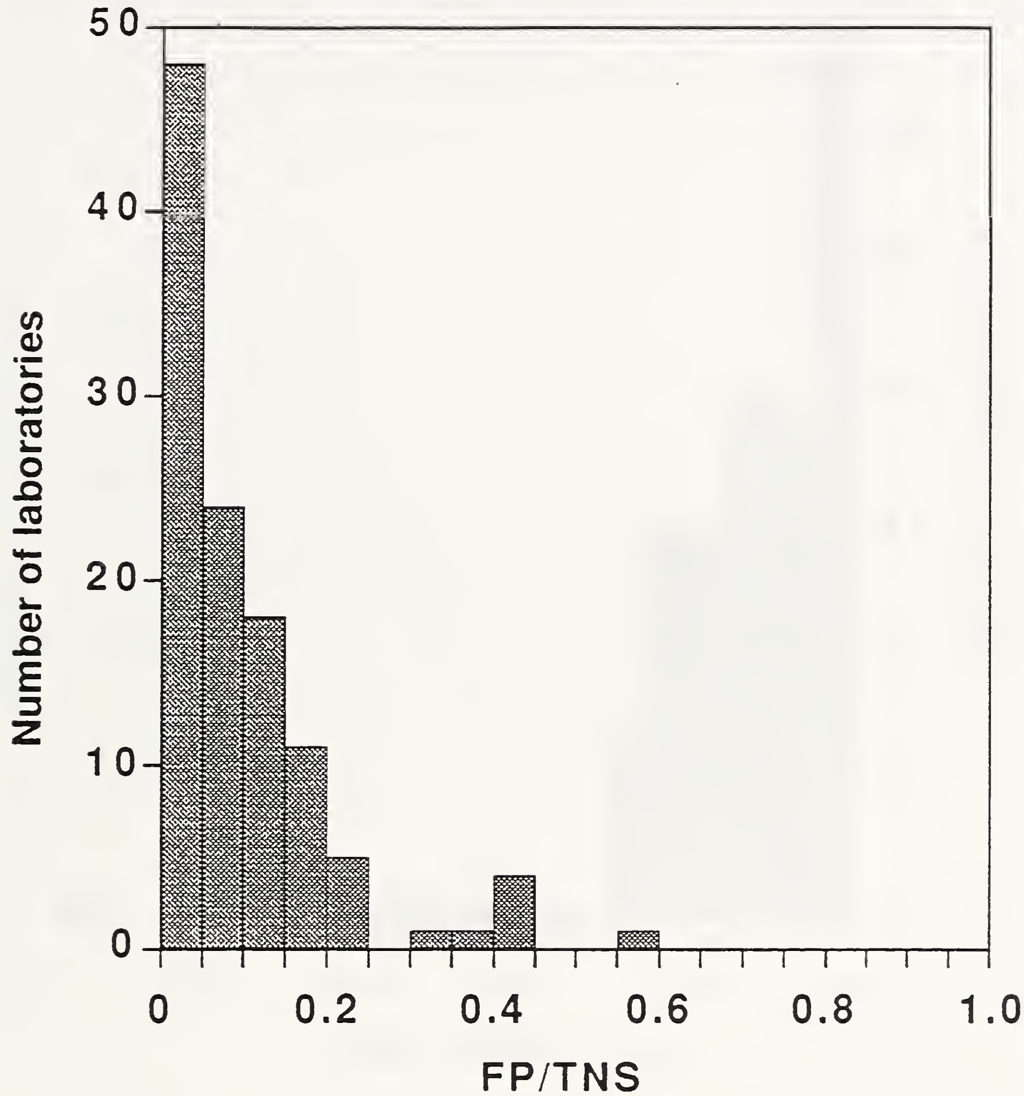


Figure 4. Histogram of the distribution of values obtained for FP(false positives)/TNS(total number of structures).

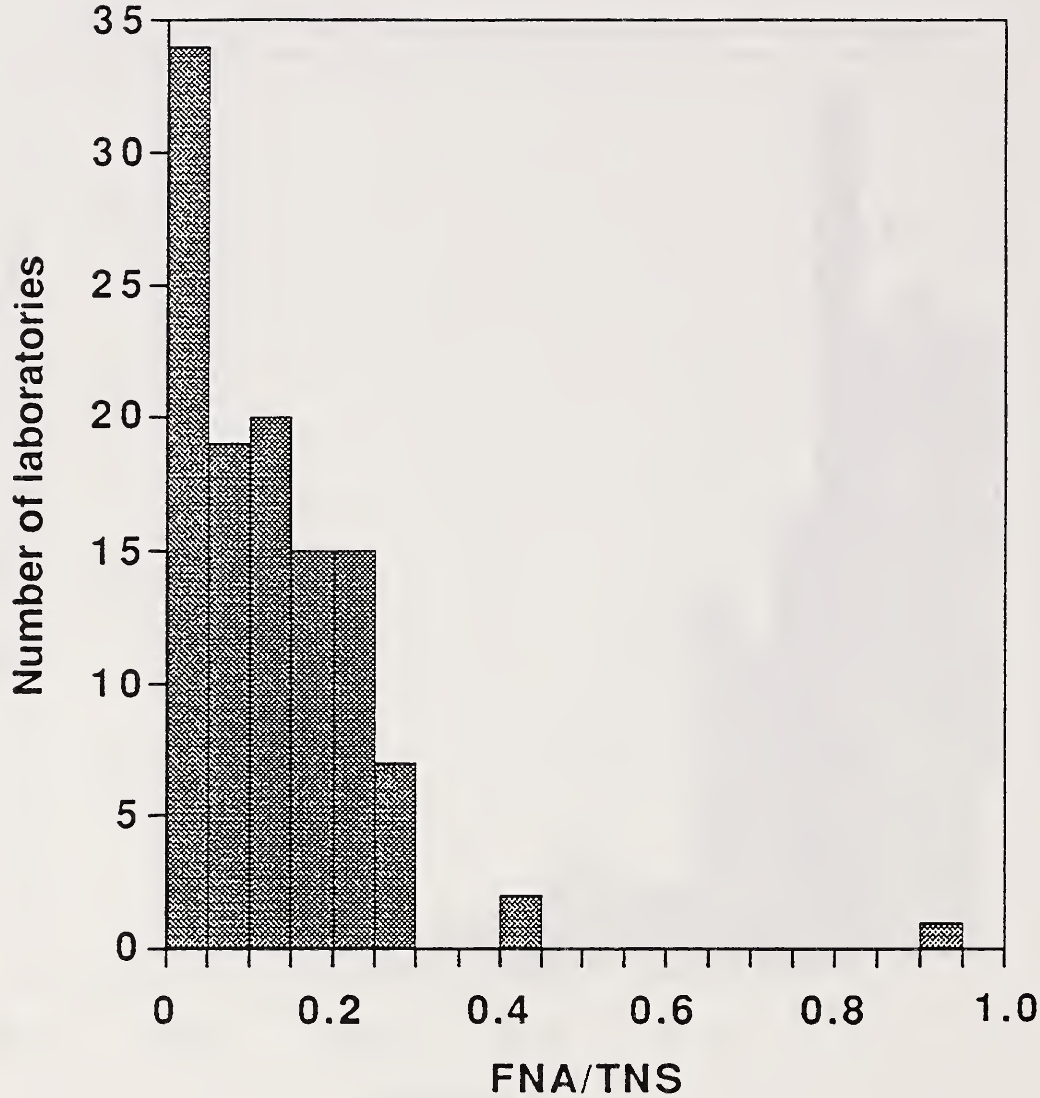


Figure 5. Histogram of the distribution of values obtained for FNA(false negatives, type A)/TNS(total number of structures).

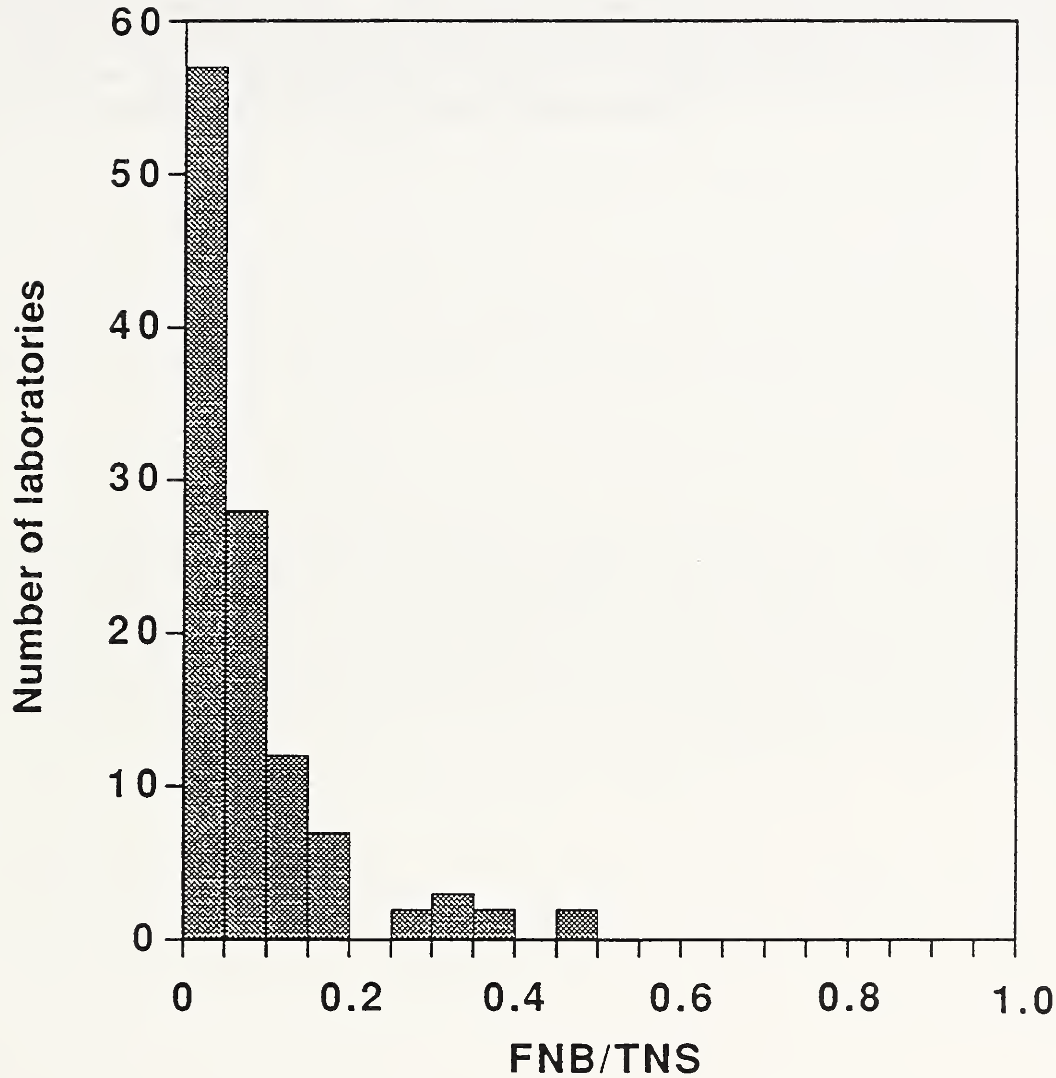


Figure 6. Histogram of the distribution of values obtained for FNB(false negatives, type B)/TNS(total number of structures).

III. Instructions for PT90-2

This proficiency test consists of two sections: 1) analysis of diffraction patterns and 2) determination of the number of structures present in images and sketches. The laboratories were divided into five groups - A, B, C, D and E and each group was sent a slightly different proficiency test. The diffraction patterns for each group were the same but the diffraction spots were labelled differently in Figures 2, 3 and 4. All of the laboratories were sent the same images and sketches but each group was given a different set of counting rules. This chapter contains the instructions, forms, and images and sketches that were the same for all the laboratories, the diffraction patterns sent to Group A laboratories and the counting protocols and associated forms sent to each of the five groups of laboratories.

Instructions for evaluation of electron diffraction patterns

Enclosed are four diffraction patterns labelled Figures 1-4. The patterns were obtained from the following materials:

Figure 1 - Evaporated gold
Figure 2 - "Unknown"
Figure 3 - Chrysotile
Figure 4 - Tremolite-actinolite

Carefully follow the instructions below for deriving and recording values from each pattern. Any length measurements made from the diffraction patterns should be recorded in mm. D-spacings should be recorded in Å.

Figure 1

From this gold pattern, determine: 1) the camera constant and 2) the standard deviation of this value (if multiple measurements are made). The method and work done in the calculations should be recorded on Form 1. The camera constant and standard deviation must then be recorded in Form 5.

Figure 2

From this pattern, determine the two unique reflections that have largest and second largest d-spacings. Record the numbers of the reflections (using the numbers on Figure 2) on Form 5. Determine the d-spacings of these reflections. The pattern has a camera constant of approximately 74.6 mm-A using a radius measurement and 149.1 mm-A using a diameter measurement. Show method and work involved in calculating d-spacings on Form 2. Determine the angle between the reflections. Record values on Form 5.

Figure 3

For this chrysotile diffraction pattern, determine the d-spacings for the reflections labelled 1-6 in Figure 3. The pattern has a camera constant of approximately 74.6 mm-A using a radius measurement and 149.1 mm-A using a diameter measurement. Show method and work involved in calculating d-spacings on Form 3. Determine the (hkl) values for these reflections and record the information on Form 5.

Figure 4

For this tremolite-actinolite diffraction pattern, the following must be determined: 1) the d-spacings for the reflections labelled 1-6 (on Figure 4), 2) the (hkl) values for these reflections and 3) the zone axis orientation of this pattern. The pattern has a camera constant of approximately 77.5 mm-A using a radius measurement and 155.0 mm-A using a diameter measurement. Show the method and work involved in calculating d-spacings on Form 4. Also describe on Form 4 how the zone axis was determined. The resulting values must be recorded on Form 5. If there any ambiguities in the derived values for the (hkl) of the reflections or the zone axis, attach a sheet to Form 4 and describe the alternative interpretation.

Form 1

Lab code _______ 02-D144

1) Describe the method used for calculating the camera constant from the pattern in Figure 1:

2) Show work done to calculate the camera constant (list any measured distances).

Form 2

Lab code ______02-D144

1) Show measurements and work involved in calculating d-spacings from the pattern in Figure 2.

Form 3 Lab code ______ 02-D144

1) Show measurements and work involved in calculating d-spacings from the pattern in Figure 3.

Form 4

Lab code ______ 02-D144

1) Show measurements and work involved in calculating d-spacings from the pattern in Figure 4.

2) Describe how the zone-axis orientation of diffraction pattern in Figure 4 was determined.

Form 5

Lab code _______ 02-D144

Figure 1

Camera Constant (mm-Å)	
Standard Deviation	

Figure 2

number of reflection with largest d-spacing (from Figure 2)	
number of reflection with second largest d-spacing (from Fig. 2)	
d-spacing for first reflection	
d-spacing for second reflection	
angle between the two reflections	

Form 5 (continued)

Lab code _____ 02-D14

Figure 3

Reflection #	d-spacing (Å)	(hkl)
1		
2		
3		
4		
5		
6		

Figure 4

Reflection #	d-spacing (Å)	(hkl)
1		
2		
3		
4		
5		
6		

Zone axis orientation	

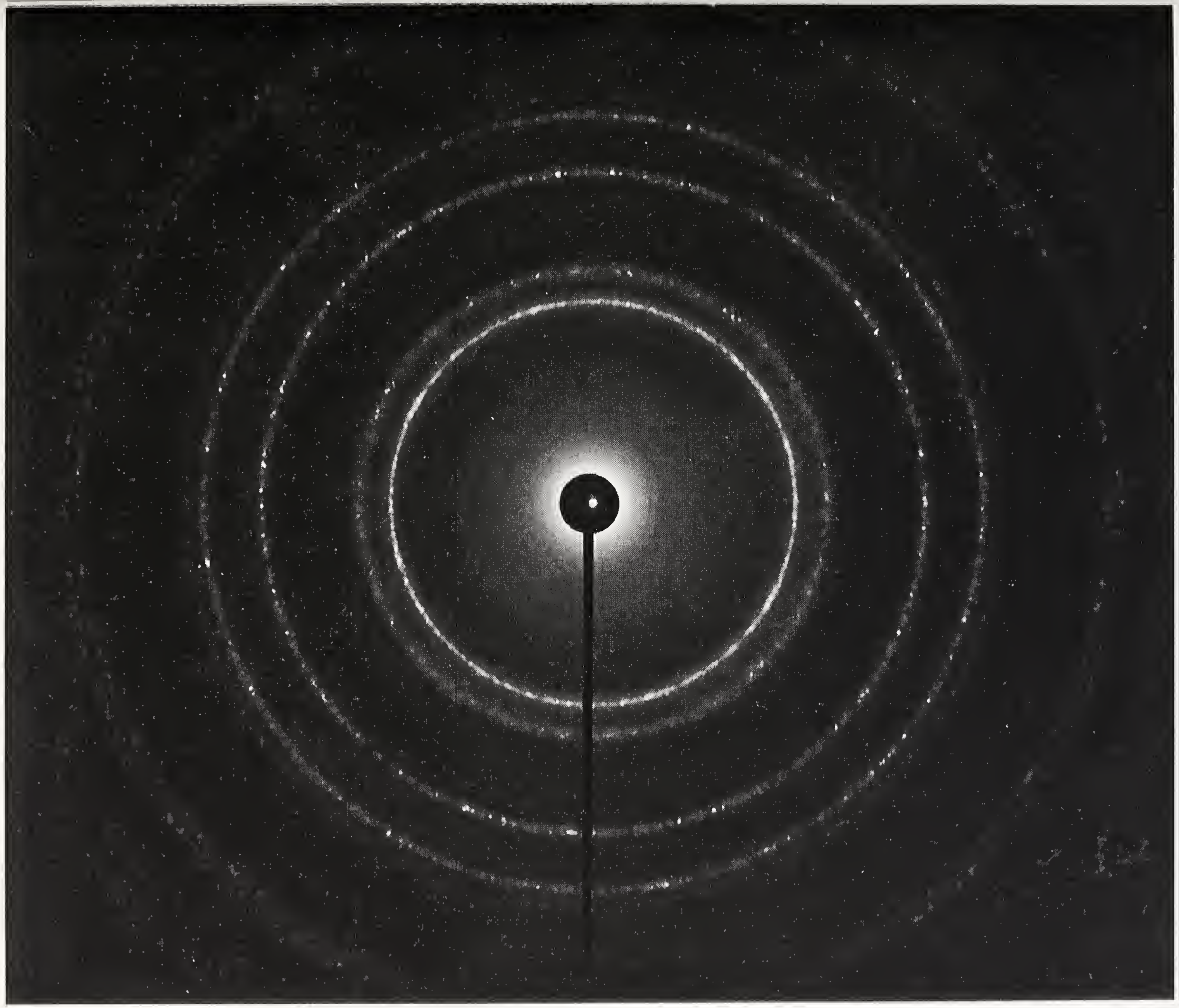


Figure 1. Diffraction pattern from an evaporated gold sample.

Figure 2. Diffraction pattern from an "unknown". The numbers are directly above the corresponding reflections.

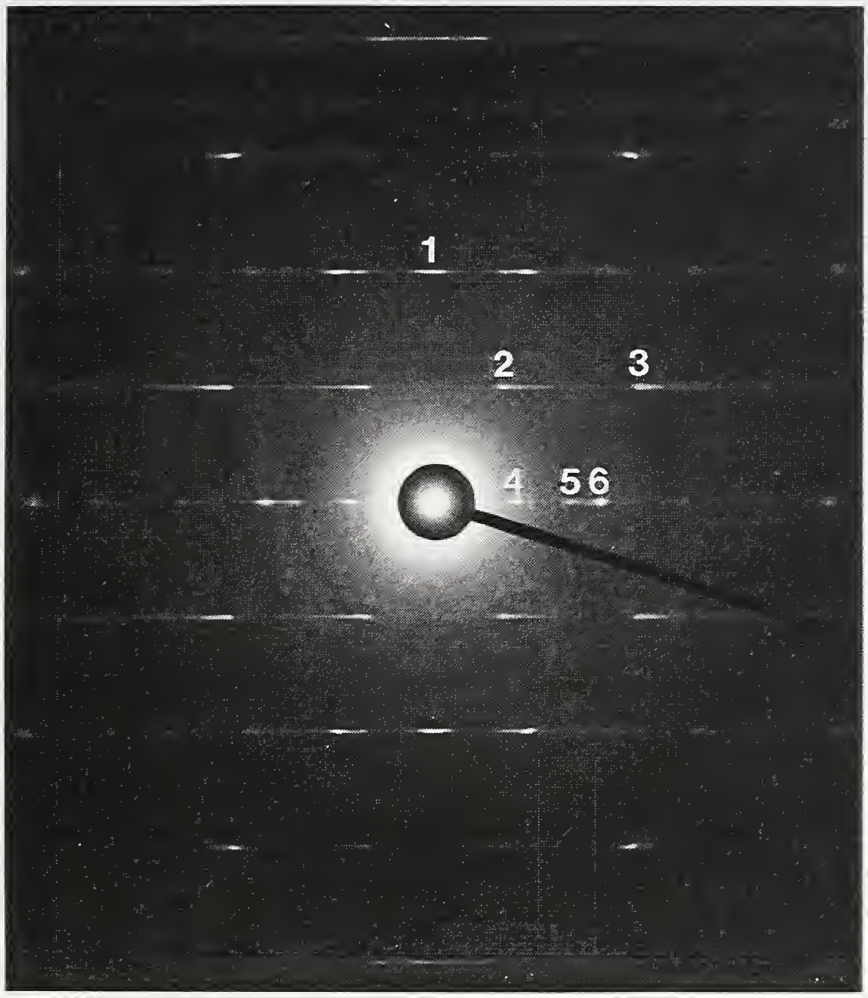


Figure 3. Diffraction pattern from chrysotile. The numbers are directly above the corresponding reflections.

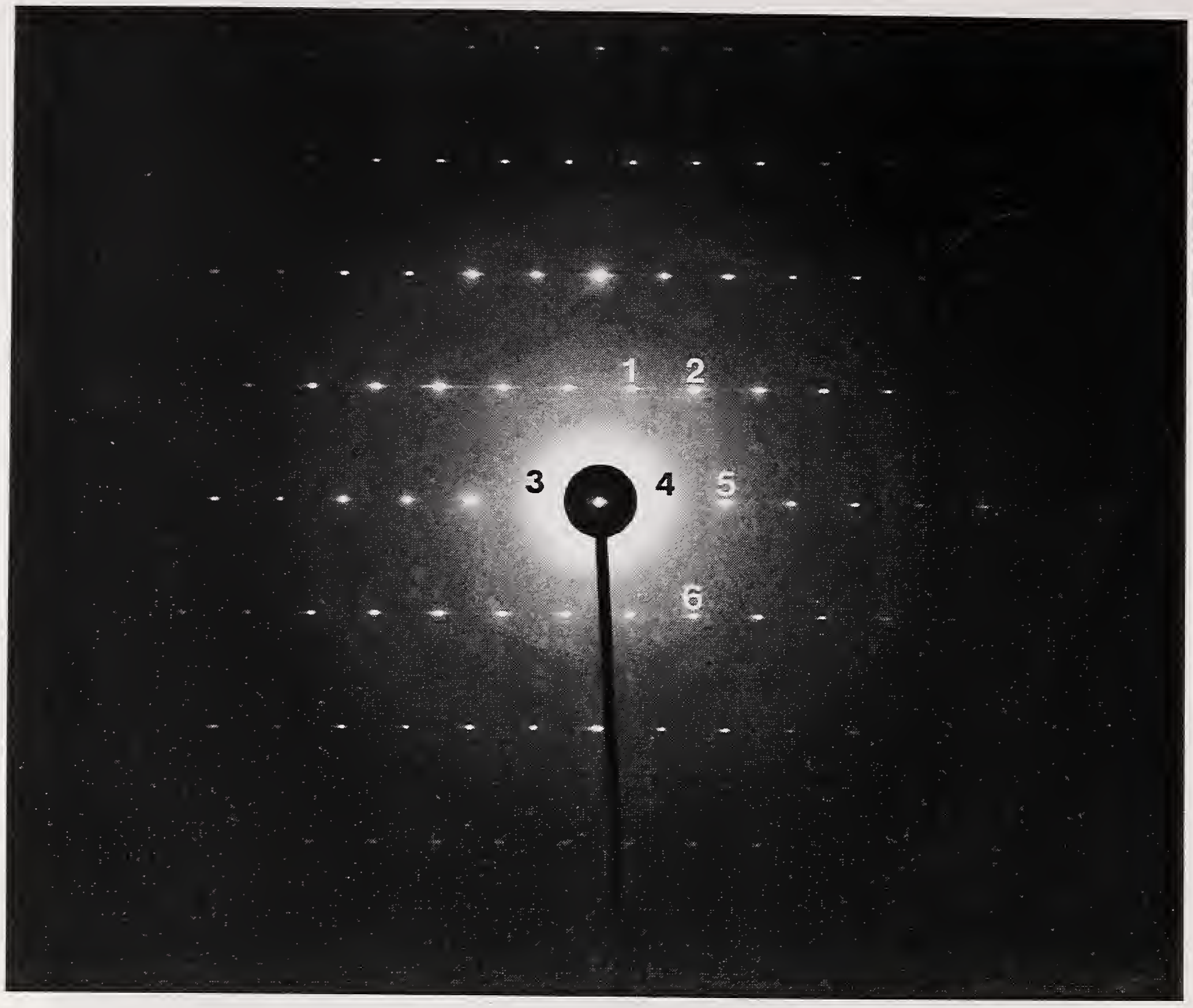


Figure 4. Diffraction pattern from an amphibole from the tremolite-actinolite series. The numbers are directly above the corresponding reflections.

Proficiency test 90-2 Part 2 - Structure counting

Instructions for structure counting

Enclosed are five sets of structure arrangements - each set taken from a different grid square. Four of the sets consist of copies of prints and the fifth set consists of sketches of structure arrangements. The structures are labelled with a grid square and a field of view. Also enclosed is a set of counting rules labelled either protocol A, B, C, D or E. For this part of the proficiency test, the laboratories are to follow the counting rules and record the field of view, a sketch and the number of structures per field of view (and any other requested information) on Form 6. A separate Form 6 should be used for each grid square. The results are to be summarized on Form 7.

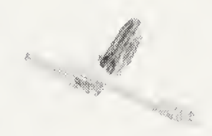
The scale of the copies and sketches is 1 cm = 0.56 micrometers. In the copies and sketches, consider all rectangular objects or curved fibers with parallel sides to be chrysotile and all irregularly shaped objects to be nonasbestos.

Please be careful in recording information on various forms. Carefully check the field of view label for each structure arrangement.

On Form 8, list any ambiguities with this set of counting rules. List any suggestions for improving this set of counting rules (attach more sheets if necessary).

Optional: If you can derive a set of counting rules that you consider an improvement over the present AHERA rules and the enclosed counting protocol, describe them on Form 9 (attach more sheets if necessary).

Grid Square D18, Field of View 1



Grid Square D18, Field of View 4

Grid Square D18, Field of View 2

Grid Square D18, Field of View 3

Grid Square D18, Field of View 7

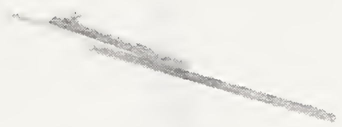
Grid Square D18, Field of View 6

Grid Sauare D18, Field of View 5

Grid Square D18, Field of View 8

Grid Square D18, Field of View 9

Grid Square A19, Field of View 1



Grid Square A19, Field of View 3

Grid Square A19, Field of View 2

Grid Square A19, Field of View 5

Grid Square A19, Field of View 4

Grid Square A14, Field of View 3

Grid Square A14, Field of View 2

Grid Square A14, Field of View 1

Grid Square A14, Field of View 4

Grid Square A14, Field of View 6

Grid Square A14, Field of View 5

Grid Square A14, Field of View 8

Grid Square A14, Field of View 7

Grid Square A14, Field of View 11

Grid Square A14, Field of View 10

Grid Square A14, Field of View 9

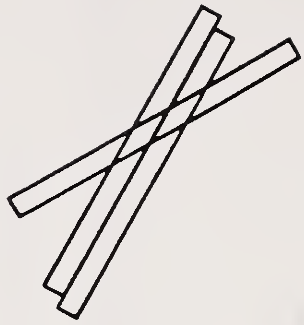
Grid Square B11, Field of View 2

Grid Square B11, Field of View 3

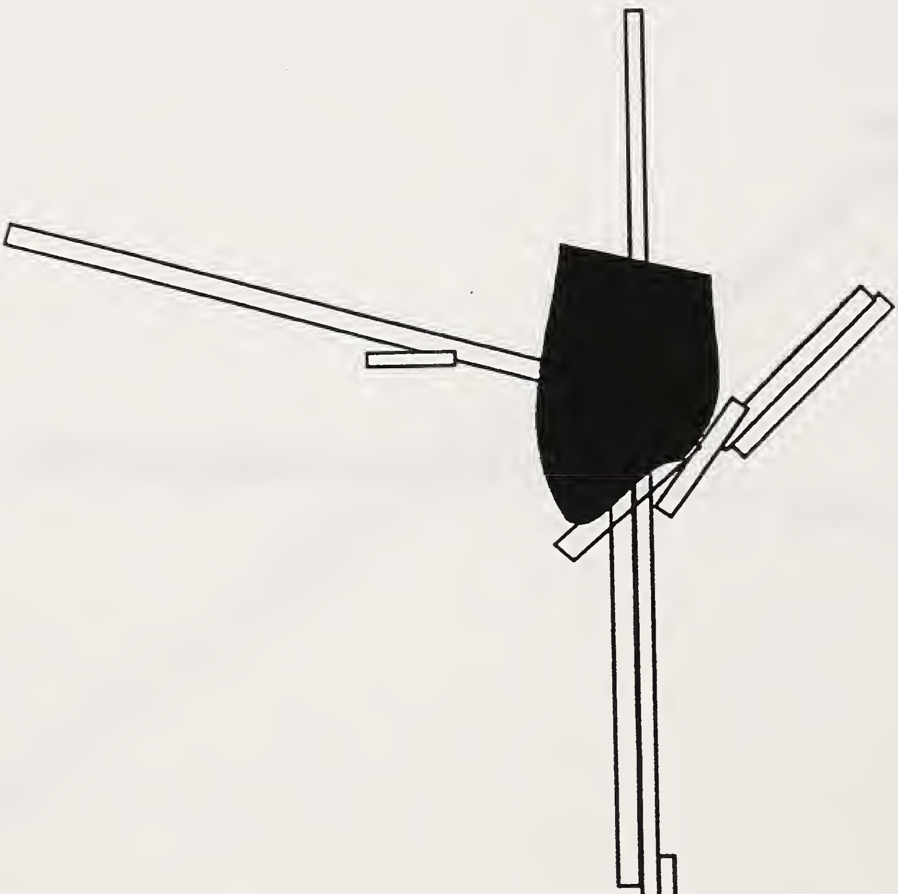
Grid Square B11, Field of View 1

Grid Square B11, Field of View 5

Grid Square B11, Field of View 4

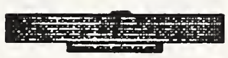


Grid square: B17
Field of view: 2



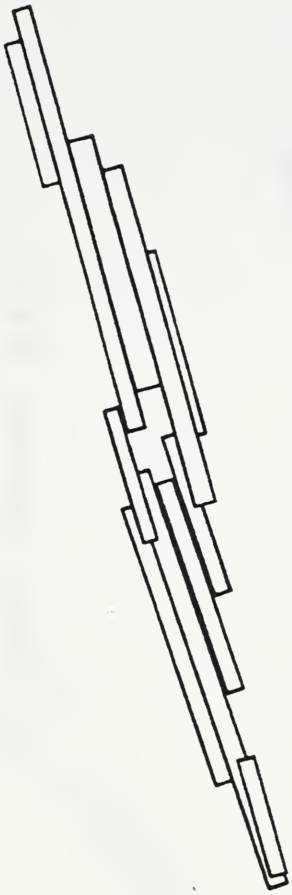
Grid square: B17
Field of view: 1

Grid square: B17
Field of view: 4



Grid square: B17
Field of view: 3

Grid square: B17
Field of view: 5

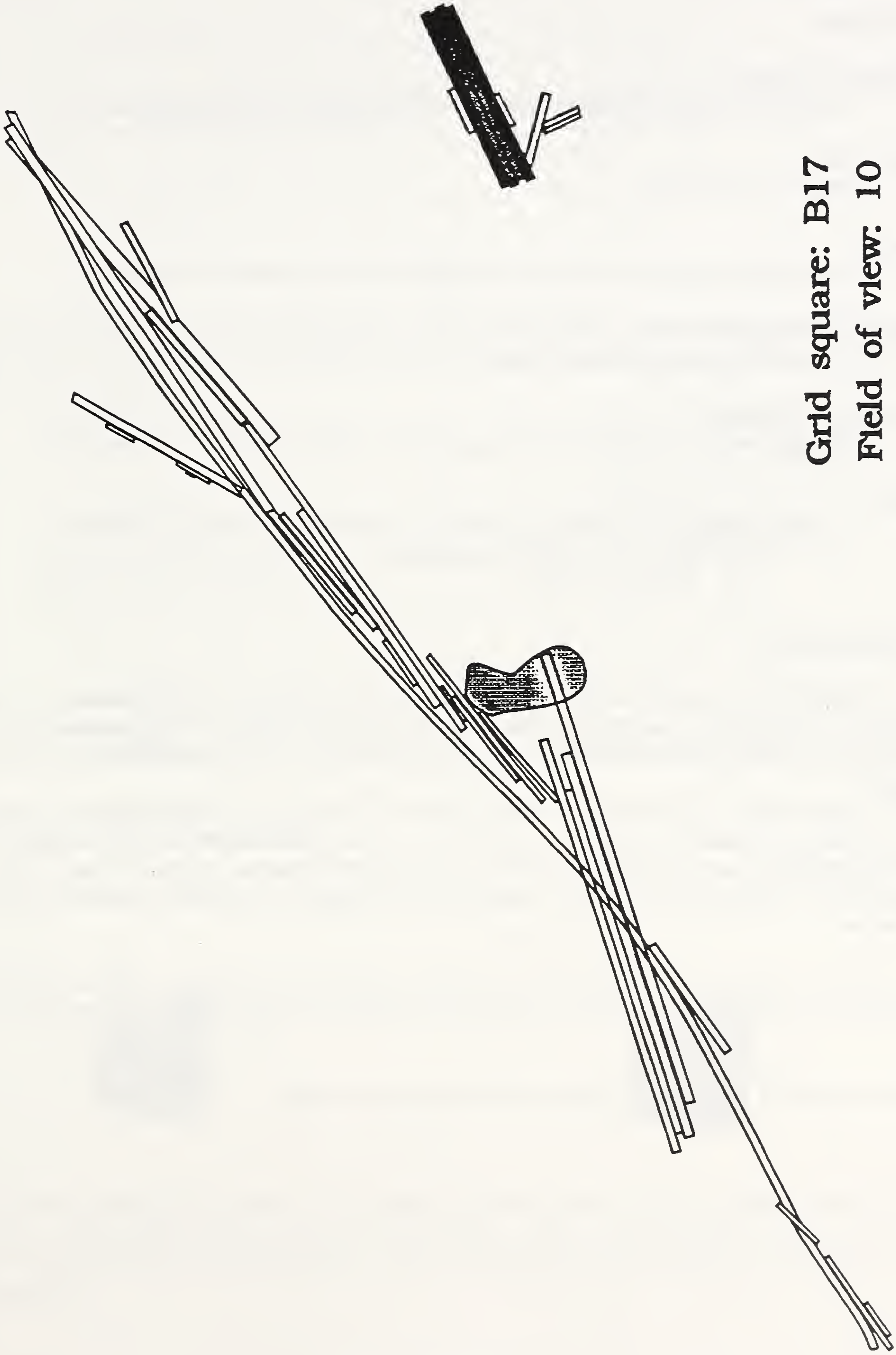


Grid square: B17
Field of view: 7

Grid square: B17
Field of view: 6

Grid square: B17
Field of view: 8

Grid square: B17
Field of view: 9



Grid square: B17
Field of view: 10

Counting Rules - Protocol A

Definitions:

Asbestos Structure:
a single fibril or a contiguous structure that contains at least one fibril

Aspect Ratio:
length to width ratio

Bundle:
a structure composed of two or more fibrils that are parallel and adjacent

Contiguous Asbestos Fibrils:
a series of touching asbestos fibrils

Contiguous Structure:
an agglomerate or series of touching particles (either asbestos or nonasbestos)

Fibril:
a single crystal of a regulated (asbestos) mineral that has an aspect ratio $\geq$5:1
Note: there are no length restrictions

Counting Rules:

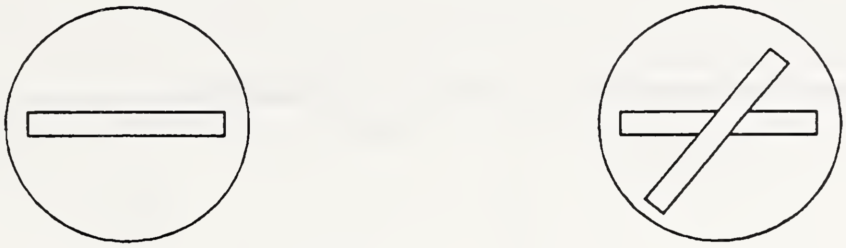
1. A single fibril is counted as one asbestos structure if the fibril $\geq$ 0.5 micrometers. A contiguous structure is counted as one asbestos structure if it contains a fibril $\geq$ 0.5 micrometers or it contains contiguous asbestos fibrils that have an overall dimension $\geq$ 0.5 micrometers (see examples next page).

2. Counting of bundles represents a special case. It is often difficult to observe the 5:1 ratio of individual fibrils due to overlap. Therefore, if a regulated asbestos mineral (of any aspect ratio) is identified in a structure that has the appearance of a bundle, the bundle is counted as one asbestos structure.

3. Asbestos structures touching a grid bar shall be counted as one-half of an asbestos structure. If the structure touches two grid bars, it shall be counted as one-third of an asbestos structure.

See examples of the counting rules on the following two pages.

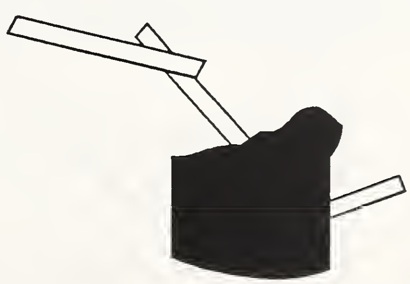
Examples of Counting Rules - Protocol A



The above structures should not be counted. The overall length of the single fibril or contiguous asbestos fibrils is less than 0.5 micrometers (the circle is 0.5 micrometers in diameter - this scaling applies to this page only).

1 structure 1 structure

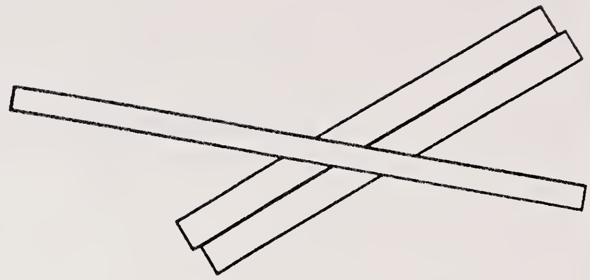
The above structures should be counted. The overall length of the single fibril or contiguous asbestos fibrils is greater than 0.5 micrometers.



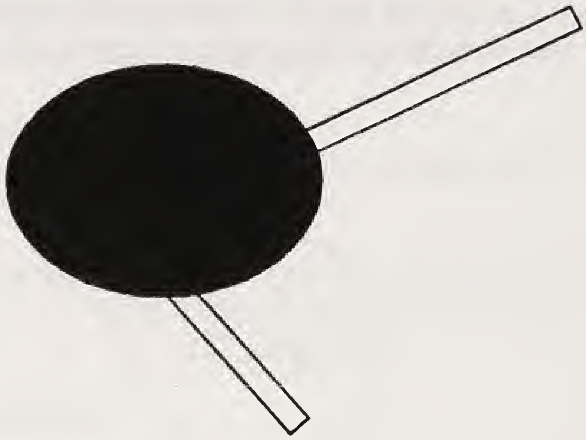
The structure to the left counts as zero asbestos structures (there are no contiguous asbestos fibrils that have a dimension greater than 0.5 micrometers). The structure to the right counts as one asbestos structure (the overall length of the contiguous asbestos fibrils is greater than 0.5 micrometers).

Protocol A - continued

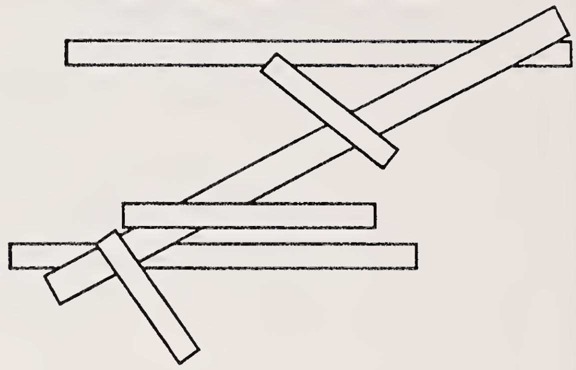
Count as 1



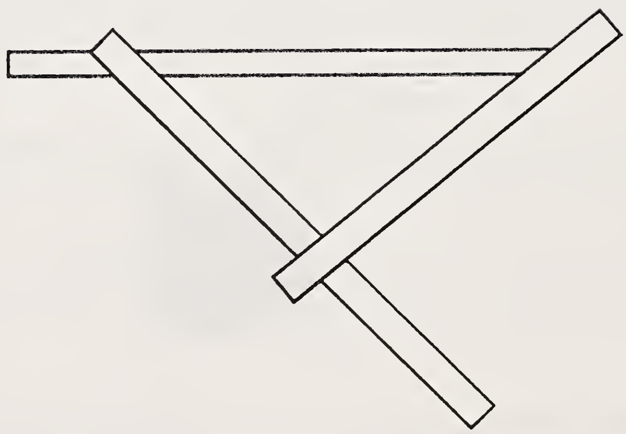
Count as 1



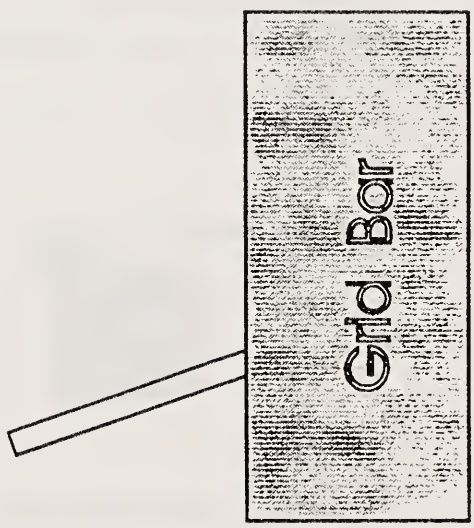
Count as 1



Count as 1



Count as 1



Count as 1/2

Form 6 - Recording form for Counting Protocol A

Lab code ________

Grid square ________

Field #	Structure sketch	Number of countable structures

Form 7 - Summary of counts for counting protocol A Lab code _______

Record in the table below the total number of countable structures found for each grid square.

Grid square	Total number of countable structures

Counting Rules - Protocol B

Definitions:

Asbestos Structure:
a single fibril or grouping of particles that contains at least one fibril

Aspect Ratio:
length to width ratio

Bundle:
a structure composed of two or more fibrils that are parallel and adjacent

Contiguous Asbestos Fibrils:
a series of touching asbestos fibrils

Contiguous Structure:
an agglomerate or series of touching particles (either asbestos or nonasbestos)

Fibril:
a single crystal of a regulated (asbestos) mineral that has an aspect ratio $\geq$ 5:1.
Note: there are no length restrictions.

Counting Rules:

1. A single fibril is counted if the fibril $\geq$ 0.5 micrometers. A contiguous structure is counted only if it contains a fibril $\geq$ 0.5 micrometers or if it contains contiguous asbestos fibrils that have an overall dimentsion $\geq$ 0.5 micrometers.

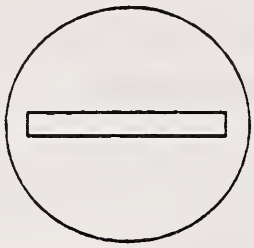
2. If an isolated fibril is identified, it is counted as one asbestos structure. If up to six fibrils are identified in a contiguous structure, they are individually counted as asbestos structures. If more than six fibrils are identified, the contiguous structure is given a value of six asbestos structures.

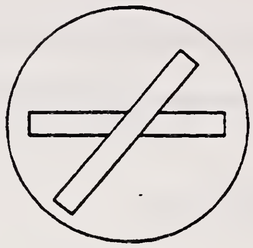
3. Counting of bundles represents a special case. It is often difficult to observe the 5:1 ratio of individual fibrils due to overlap. Therefore, if a single crystal of a regulated asbestos mineral (of any aspect ratio) is identified in a structure that has the appearance of a bundle, it is counted as one asbestos structure.

4. Asbestos structures touching a grid bar shall be counted as one-half of an asbestos structure. If the structure touches two grid bars, it shall be counted as one-third of an asbestos structure.

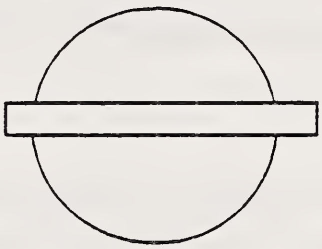
See examples on next two pages.

Examples of Counting Rules - Protocol B

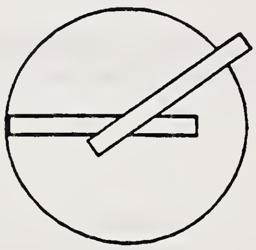




The above structures should not be counted. The overall length of the single fibril or contiguous asbestos fibrils is less than 0.5 micrometers (the circle is 0.5 micrometers in diameter - this scaling applies to this page only).



1 structure



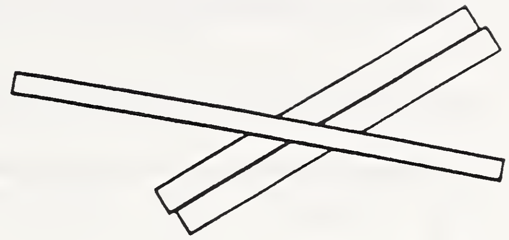
2 structures

The above structures should be counted. The overall length of the single fibril or contiguous asbestos fibrils is greater than 0.5 micrometers.

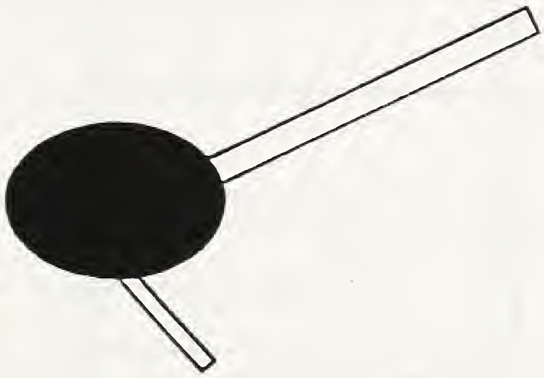
The structure to the left counts as zero asbestos structures (there are no contiguous asbestos fibrils that have a dimension greater than 0.5 micrometers). The structure to the right counts as three asbestos structures (the overall length of the contiguous asbestos fibrils is greater than 0.5 micrometers and therefore every fibril present is counted).

Protocol B - continued

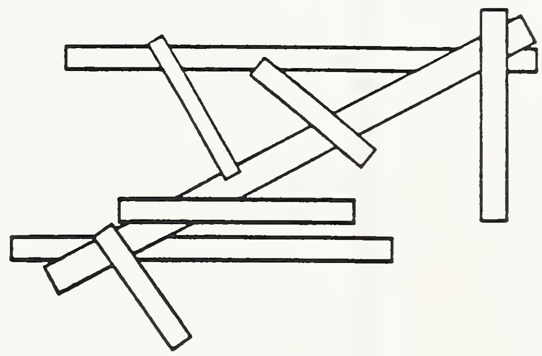
Count as 1



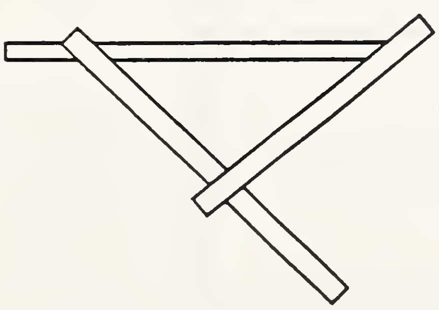
Count as 3



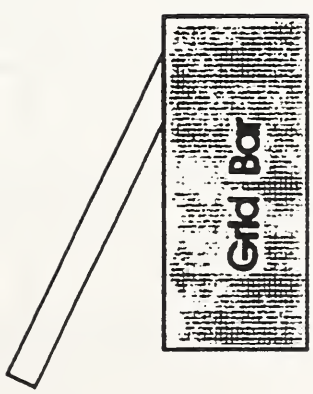
Count as 2



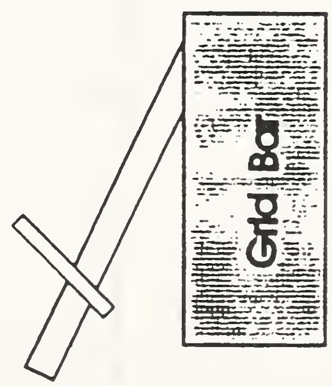
Count as 6



Count as 3



Count as 1/2



Count as 1

Form 6 - Recording form for Counting Protocol B

Lab code _______

Grid square _______

Field #	Structure sketch	Number of countable structures

Form 7 - Summary of counts for counting protocol B Lab code _______

Record in the table below the total number of countable structures found for each grid square.

Grid square	Total number of countable structures

Counting Rules - Protocol C

This protocol is part of a proposed draft method for the International Standards Organization.

Definitions:

Fiber

Any particle with parallel or stepped sides, with an aspect ratio of 5:1 or greater, shall be defined as a fiber. For chrysotile asbestos, the single fibril shall be defined as a fiber. A fiber with stepped sides shall be assigned a width equal to the average of the minimum and maximum widths. This average shall be used as the width in determination of the aspect ratio.

Bundle

A grouping composed of apparently attached parallel fibers shall be counted as a bundle of a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may be any value, provided that the individual constituent fibers has an aspect ratio of ≥5:1.

Cluster

An aggregate of randomly oriented fibers, with or without bundles, shall be defined as a cluster. Clusters occur as two varieties:

Cluster type A: a disperse and open network, in which both ends of individual fibers and bundles can be separately identified and their dimensions measured. If the cluster consists of up to 5 such fibers or bundles, the individual fibers and bundles comprising the cluster shall be separately counted and measured, and recorded as individual fibers and bundles. If the cluster consists of more than 5 fibers or bundles it shall be counted and measured in the same manner as a cluster type B.

Cluster type B: a complex and tightly bound network, in which one or both ends of each individual fiber or bundle are obscured, such that the dimensions of individual fibers cannot be measured. In this case the cluster shall be recorded as single cluster, and the overall dimensions in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

The recording of clusters shall be based on the predominant characteristics of the structures. For example, a cluster consisting predominantly of 4 fibers, but with smaller regions of attached material containing fibers, shall be considered as a type A cluster of 4 fibers to be recorded separately as fibers. The procedure for treatment of clusters is illustrated by examples in Figures C1 and C2.

Matrix

a fiber, fibers, or bundles, may be attached to, or partially concealed by, a single particle or group of overlapping non-fibrous particles. This structure shall be defined as a matrix. Three types of matrices can be defined:

Matrix type A: a structure consisting of an equant particle or linked group of particles, linked by fibers or bundles in which both ends of fibers or bundles can be located approximately. If the matrix consists of up to 5 such fibers or bundles, the fibers or bundles shall be recorded as separate fibers or bundles. If the matrix consists of more than 5 fibers or bundles, it shall be counted and measured in the same manner as a matrix type B.

Matrix type B: a structure consisting of an equant particle or linked group of particles, in which the ends of fibers or bundles project from the particles, but the other ends of the fibers or bundles are obscured. Fibers and bundles shall be treated differently, depending on whether the obscured length could not possibly be more than one third of the total length. If the matrix exhibits up to 5 fibers or bundles, those fibers for which the obscured length could not be more than one third of the total length shall be counted as separate fibers or bundles. The residual matrix, if it exhibits additional fiber terminations which cannot be separately counted because the unobscured lengths are too short, shall be recorded as one matrix. The overall dimensions of each matrix in the two perpendicular directions defining the maximum and minimum dimensions shall be recorded.

Matrix type C: a structure in which fibers can be seen and identified in the interior, but which incorporates no fibers which project from the outside edges. This type of matrix can originate as a result of partial dissolution of binders during specimen preparation, when the original particle was a composite material containing asbestos. This type of matrix shall be recorded as a single matrix. The overall dimensions of the matrix in the two perpendicular dimensions defining the maximum and minimum dimensions shall be recorded.

In practice, structures can occur in which different areas exhibit features of the three types of matrix. In this case, the predominant characteristic of the structure should be determined, and then a logical procedure should be followed, in which the fibers are enumerated first, followed by assignment of the remaining structures according to the counting rules. Examples of the procedure which shall be followed are shown in Figures C1 and C3.

Fibers intersecting grid bars:

Count a fiber which intersects a grid bar counted only for two sides of the grid opening, as illustrated in figure that follows. The length of the fiber shall be recorded as twice the unobscured length. Fibers intersecting either of the other two sides shall not be included in the count. This procedure ensures that the numerical count will be accurate, and that the best average estimate of length has been made.

Procedure for data recording

1) Fibers

On the structure counting form (Form 6), fibers, as described in the definitions section, shall be recoded by the designation "F". If the fiber is a separately-counted part of a cluster or matrix structure, a two-digit number shall be attached as a suffix, to identify all such constituent components of the cluster or matrix structure. For example, F04 shall be used to denote a fiber forming part of a structure (cluster) number 04.

2) Bundles

On the structure counting form (Form 6), bundles, as described in the definitions section, shall be recorded by the designation "B". If the bundle is a separately-counted part of a cluster or matrix structure, a two-digit number shall be attached as a suffix, to identify all such constituent components of the cluster or matrix structure. For example, B04 shall be used to denote a bundle forming part of structure (matrix) number 04.

3) Clusters

On the structure counting form (Form 6), isolated clusters, as described in the definitions section, shall be recorded by the designation "C". If a localized cluster is attached to a group of fibers or bundles, and the fibers and bundles have been counted separately because this procedure was defined by the predominant characteristics of the structure, a two-digit number shall be attached as a suffix, to identify all such constituent components of the structure. For example, C04 shall be used to denote a localized cluster forming part of structure number 04.

4) Matrices

On the structure counting form (Form 6), isolated matrices, as described in the definitions section, shall be recorded by the designation "M". If the type B or C matrix is attached to a group of fibers or bundles, and the fibers have been counted separately because this procedure was defined by the predominant characteristics of the structure, a two-digit number shall be attached as a suffix, to identify all constituent components of the structure. For example, M04 shall be used to denote a matrix forming part of structure number 04.

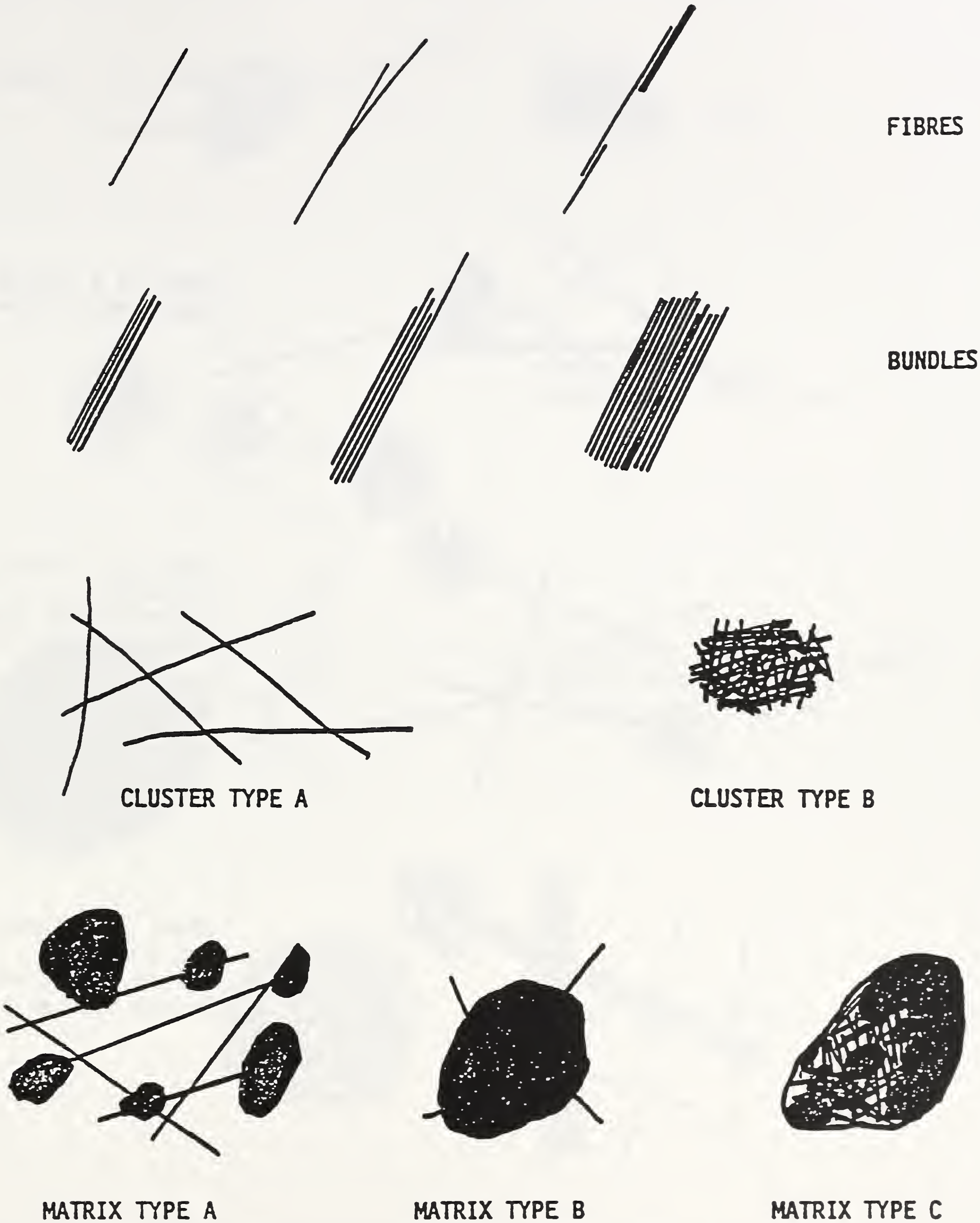
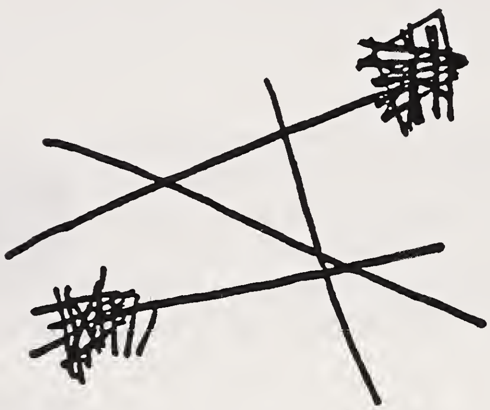


Figure C1 - Fundamental morphological structure types

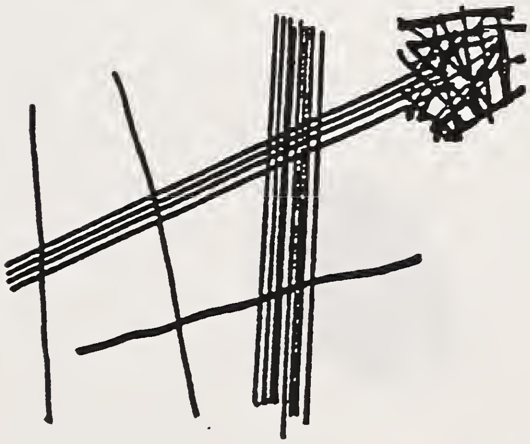
Count as 1 cluster

Record as C

Count as 5 fibres
Record each fibre as Fn



Count as 4 fibres and 2 clusters
Record fibres as Fn, and clusters as Cn

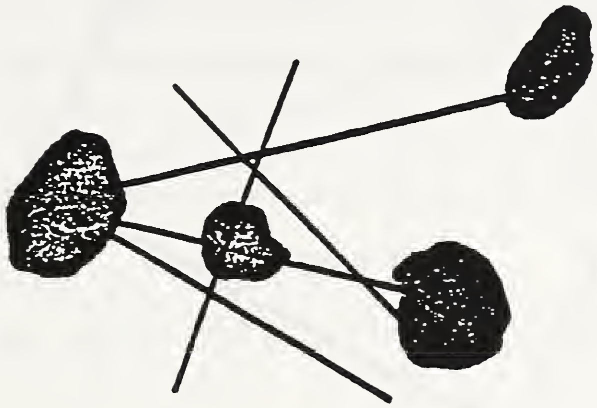


Count as 3 fibres, 2 bundles and 1 cluster
Record fibres as Fn, bundles as Bn and cluster as C

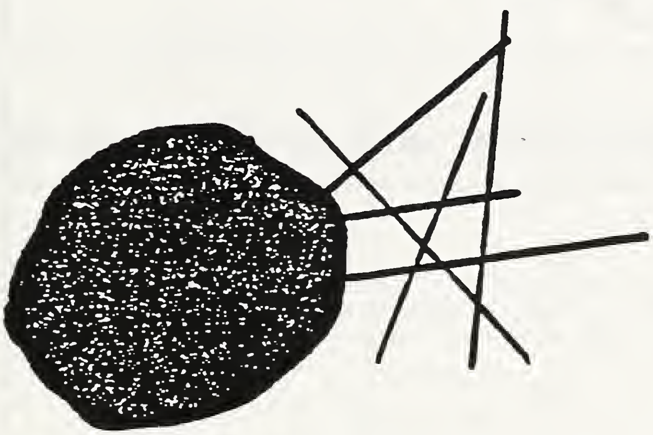
NOTE - n is a two digit number, starting at 01, assigned to each disassembled complex structure as it is encountered during the fibre count

Figure C2 - Examples of recording of complex asbestos clusters

Count as 1 fibre



Count as 5 fibres
Record each fibre as Fn



Count as 3 fibres and 1 matrix
Record each fibre as Fn
Record 1 matrix as Mn

Count as one matrix

NOTE - n is a two digit number, starting at 01, assigned to each disassembled complex structure as it is encountered during the fibre count

Figure C3 - Examples of recording of complex asbestos matrices

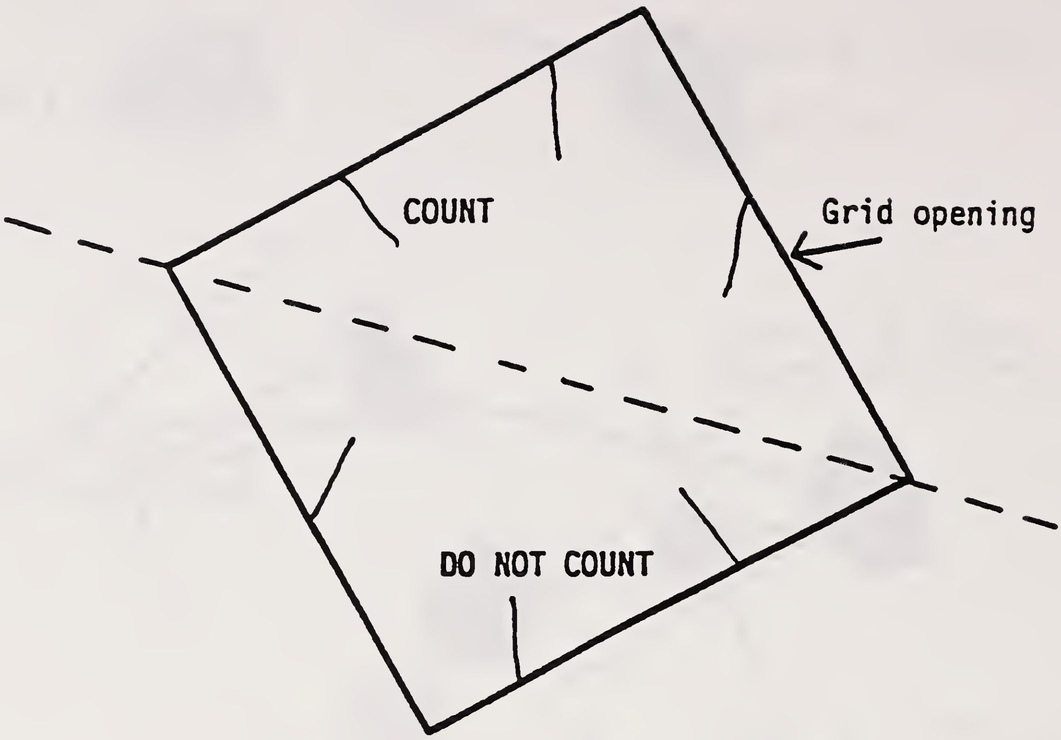


Figure C4 Counting of fibres which intersect grid bars

Form 6 - Recording form for Counting Protocol C

Lab code _______

Grid square _______

Field #	Structure sketch	Type of structure	Length (μm)	Width (μm)	Area (Length x Width)	Number of countable structures

Form 7 - Summary of counts for counting protocol C Lab code _______

Record in the table below the total number of countable structures and the sum of the areas of the countable structures for each grid square.

Grid square	Total number of countable structures	Mean of areas	Standard deviation of area measurements

Counting Rules - Protocol D

The basic counting protocol and definitions to be followed are described by the AHERA (Appendix A to subpart E--Interim Transmission Electron Microscopy Analytical Methods--Mandatory and Nonmandatory--and mandatory section to determine completion of response actions, 40 CFR part 763, October 30, 1987). A copy of the illustrations of counting rules from the Federal Register is enclosed. The AHERA rulings are to be followed unless there is a clarification or modification described in the text and illustrations that follow.

The following is an explanation and description of modifications to AHERA counting rules for NIST proficiency testing. For those laboratories that participated in the first proficiency test, additions have been made to these modifications. A summary of the additions is given the "special cases" section.

Note on countable asbestos structures:

A noncountable asbestos structure is defined as an asbestos fiber that is <0.5 μm and/or has a length:width ratio < 5:1.

Length of structures:

If a fiber has an irregularly shaped end, measure the longest length that is parallel to the fiber axis to determine if it exceeds 0.5 micrometers. (See example 1 in "special cases" section).

Intersecting fibers, bundles:

AHERA rules: The AHERA rules define an intersection as, "nonparallel touching or crossing of fibers....". A bundle is defined as a "structure composed of three or more fibers in a parallel arrangement....". A strict interpretation of these rules would imply that if bundles cross then there are multiple intersections ($\geq$ 3 intersections). Therefore the arrangement of crossing bundles would qualify as a cluster and would be counted as one structure. However, from a previous round robin study it is clear that laboratories have had many differing interpretations for these types of arrangements. The modification for NIST proficiency testing is a common-sense interpretation.

Modification of AHERA rules for NIST proficiency testing: A single intersection is defined as, "nonparallel touching or crossing of fibers (single or double) or bundles" (a double fiber consists of two parallel, touching fibers and would by itself count as one structure). This modified definition of an intersection means that an arrangement of two intersecting fibers (single or double) or two intersecting bundles count as two structures (see examples). NOTE: two fibers at a slight angle are not parallel and therefore are considered intersecting.

If a countable fiber or bundle is intersecting a bundle, but does not protrude by 0.5 micrometers it is counted as structure if the fiber or bundle can be traced for $\geq$0.5 micrometers (see example, "special case").

Nonstandard bundles:

AHERA rules: There is no ruling for nonstandard bundles.

NIST proficiency testing rule: Nonstandard bundles include those with splayed ends, those that are bent and those that contain fiber(s) that have bowed away from the main bundle. These cases will count as one bundle. Examples are attached.

Bundles and clusters containing noncountable asbestos structures:

Note: A noncountable asbestos structure is defined as an asbestos fiber that is <0.5 µm and/or has a length:width ratio $< 5:1$.

AHERA rules: These cases are not dealt with in the AHERA rules.

NIST proficiency testing rule: If the noncountable asbestos structure is touching and parallel to a 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 bundle or fiber, it is treated as if it were not present. Examples follow.

Fibers, bundles, clusters on grid bars:

AHERA rules: There is no ruling for fibers, bundles or clusters on grid bars.

NIST proficiency testing rule: A fiber (single or double), bundle or cluster touching a grid bar is counted the same way it would be if the grid bar was not there (see examples).

Closely spaced parallel bundles and fibers:

AHERA rules: The fibers or bundles are counted as separate fibers or bundles if they are separated by more than a fiber diameter. This rule is ambiguous for separated fibers where there are fibers of different diameters. This rule is ambiguous for separated bundles because it is unclear what fiber in the two bundles to use for the diameter measurement.

Modification of AHERA rules for NIST proficiency testing: If a space can be seen between the two parallel fibers or bundles, count them as two separate structures (see examples).

Asbestos associated with nonasbestos particles - matrices and others:

AHERA rules: A matrix is defined as, "fiber or fibers with one end free and the other embedded in or hidden by a particle. The exposed fiber must meet the fiber definition." The size of the particle is not defined.

Modification of AHERA rules for NIST proficiency testing: For proficiency testing, particles that are < 0.5µm in their longest dimension are considered coatings and do not qualify as matrix particles. Examples of counting of asbestos structures associated with particles are attached.

Special cases (see "special cases section"):

1) If a countable asbestos fiber or bundle has both ends in matrix particles, the fiber or bundle counts as one structure.

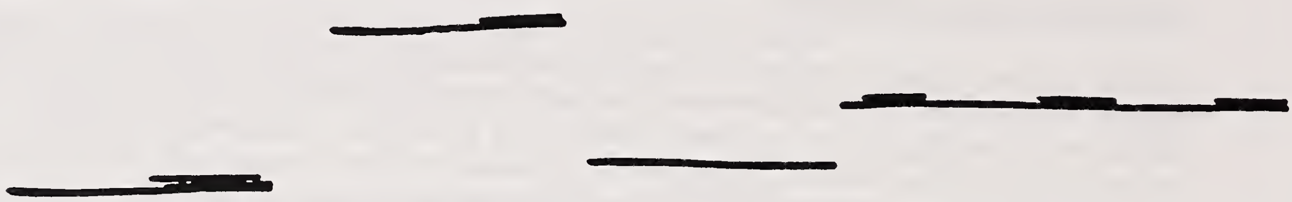
2) If a countable fiber is associated and tangential to a matrix and the two ends of the fiber are visible, it is counted as a structure.

Counting bundles and clusters:

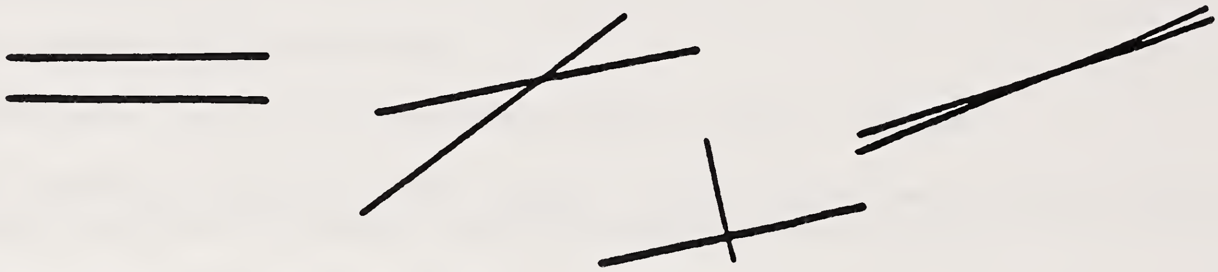
AHERA rules: A fiber is defined as, "greater than or equal to 0.5 µm in length with an aspect ratio (length to width) of 5:1 or greater and having substantially parallel sides". A bundle consists of, "three or more fibers" and a cluster consists of a random arrangement of fibers. A strict interpretation of these definitions means that a bundle or cluster must contain fibers longer than 0.5 µm and with an aspect ratio of 5:1 or greater. The dimensions and aspect ratios apply to the fibers and not the bundle or cluster itself. This definition will be followed in the NIST proficiency testing. In a previous round robin this ruling was misinterpreted by several laboratories. Examples of correct interpretations are attached.

AHERA counting guidelines used in determining number of countable asbestos structures:

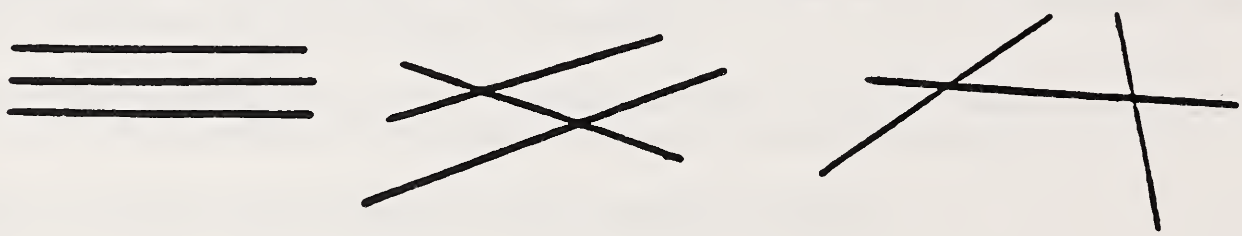
Count as 1 fiber; 1 Structure; no intersections.



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.



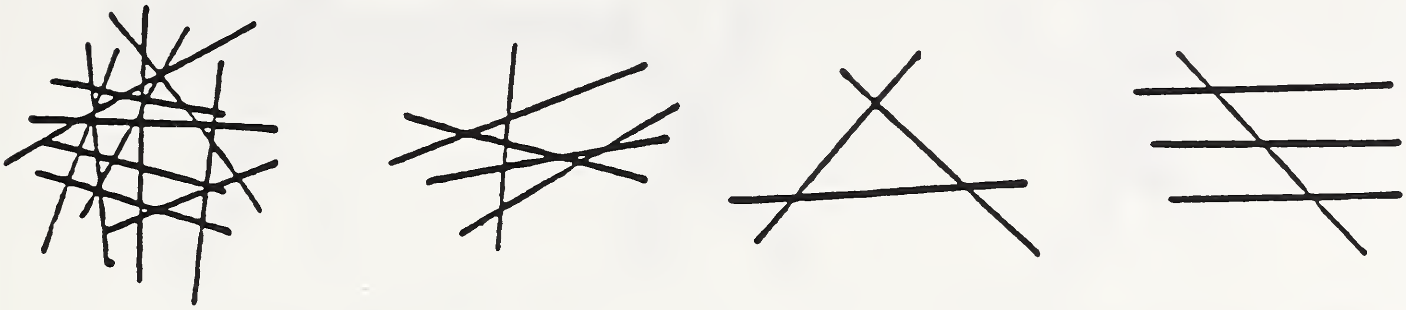
Count as 3 structures if space between fibers is greater than width of 1 fiber diameter or if the number of intersections is equal to or less than 2.



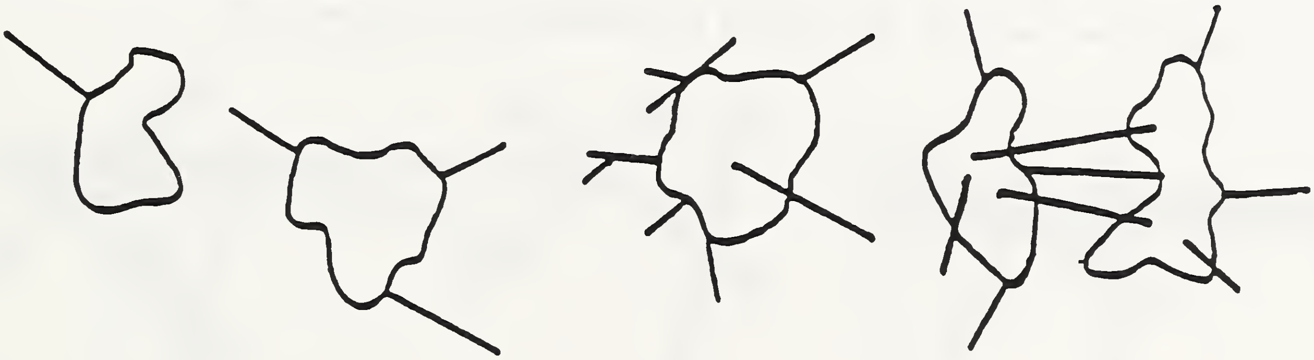
Count bundles as 1 structure; 3 or more parallel fibrils less than 1 fiber diameter separation.

AHERA counting guidelines (continued):

Count clusters as 1 structure; fibers having greater than or equal to 3 intersections.



Count matrix as 1 structure.



DO NOT COUNT AS STRUCTURES:

Fiber protrusion
<5:1 Aspect Ratio

No fiber protrusion

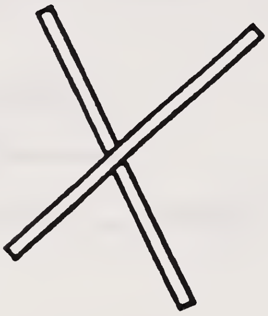
Fiber protrusion
<0.5 micrometer

— <0.5 micrometer in length

▬ <5:1 Aspect Ratio

Clarifications and modifications to AHERA counting rules to be used for TEM proficiency testing:

Intersecting fibers, bundles:

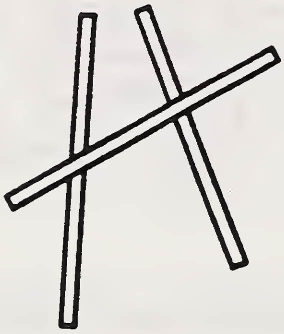


2F

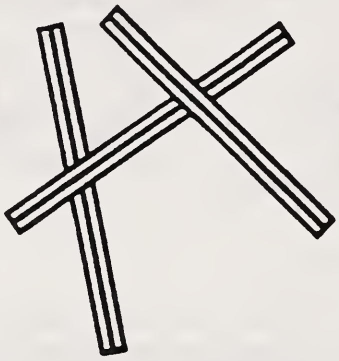
2F

1B, 1F

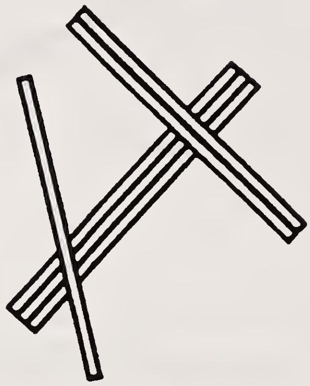
Count the cases in this row as 2 structures (1 intersection)



3F

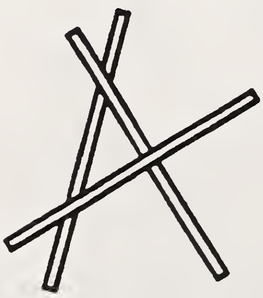


3F

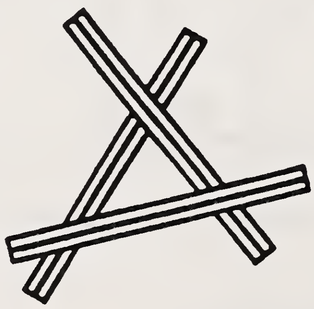


1B, 2F

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



3F



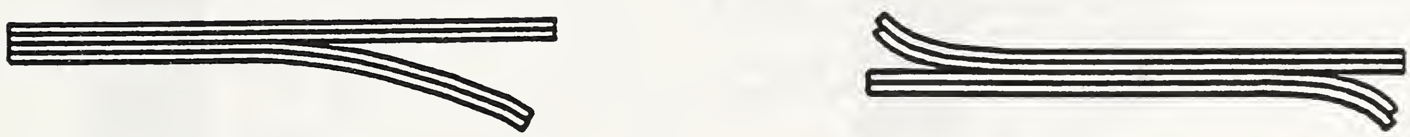
3F

Count these cases as 1 structure (3 intersections)

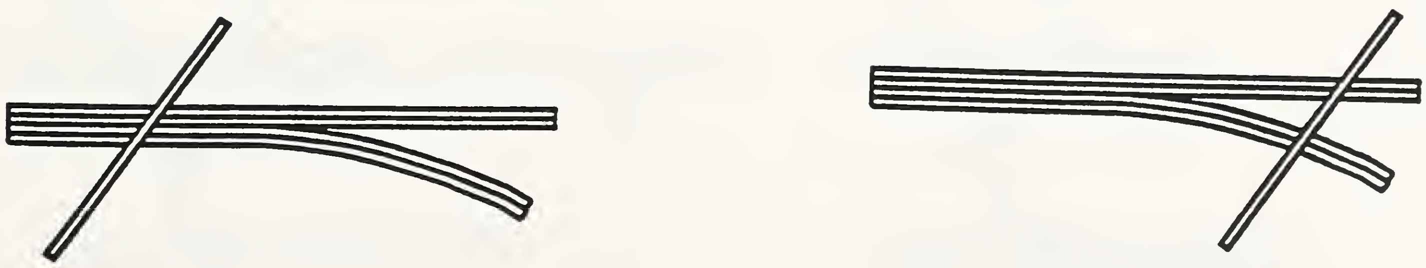
<u>Nonstandard bundles:</u>

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

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

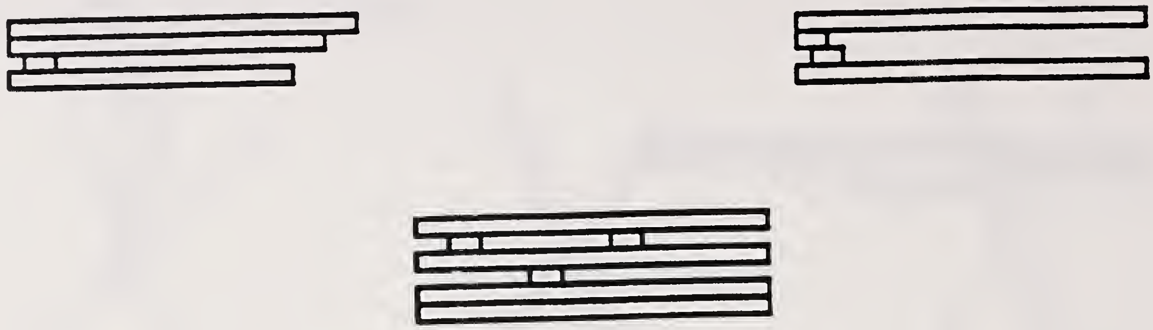


Count each in this row as 1 structure (1B)
(splayed bundles)



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

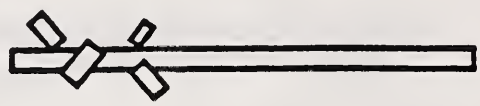
Bundles and clusters containing noncountable asbestos structures:



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

2F

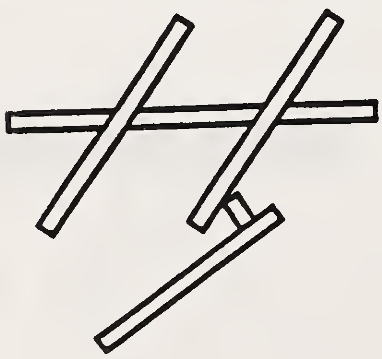
Count as 2 structures



1F

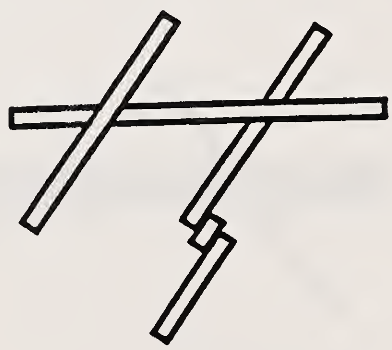
Count as 1 structure

(noncountable asbestos touching but not parallel to countable asbestos)



4F

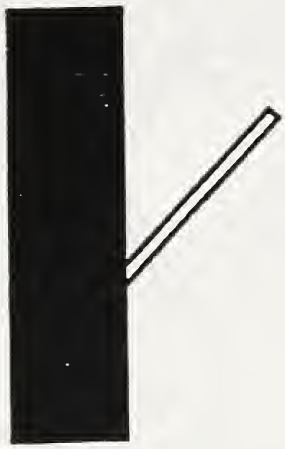
Count as 4 structures



1B, 2F

Count as 3 structures

Fibers, bundles, clusters on grid bars:



1F

1B

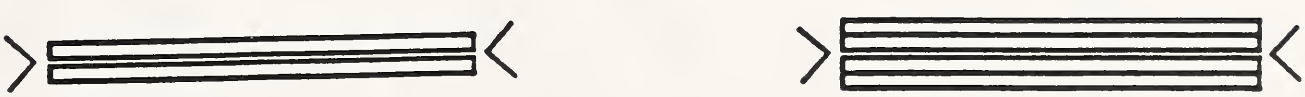
1C

Count the cases in this row as 1 structure

Count as 2 structures (2F)

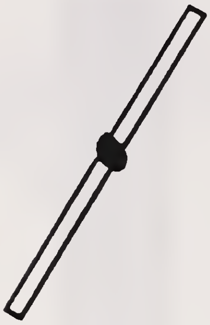
Count as 3 structures (3F)

Closely spaced parallel bundles and fibers:

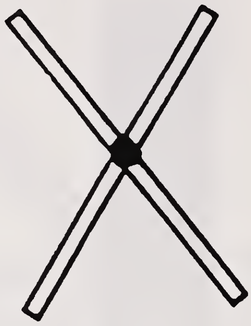


Count the cases on this row as 2 structures (2F)

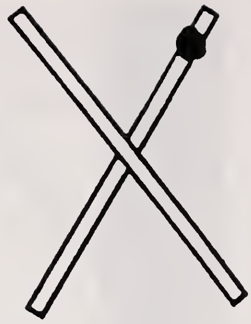
<u>Asbestos associated with nonasbestos particles:</u>



1 structure (1F)

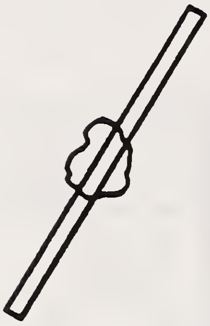


2 structures (2F)

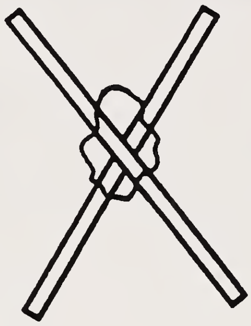


2 structures (2F)

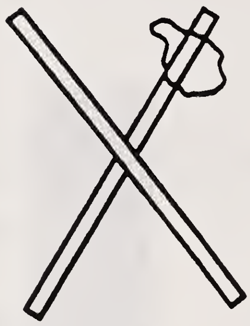
(for the above cases, the nonasbestos particle is < 0.5μm)



1 structure (1F)

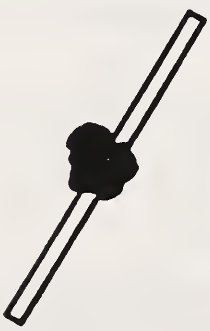


2 structures (2F)



2 structures (2F)

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



1 structure (1M)

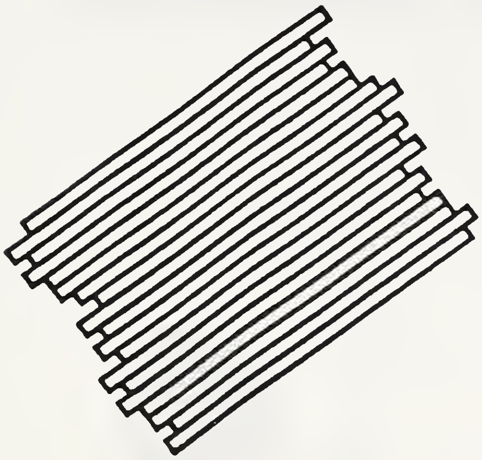
1 structure (1M)

2 structures (1M, 1F)

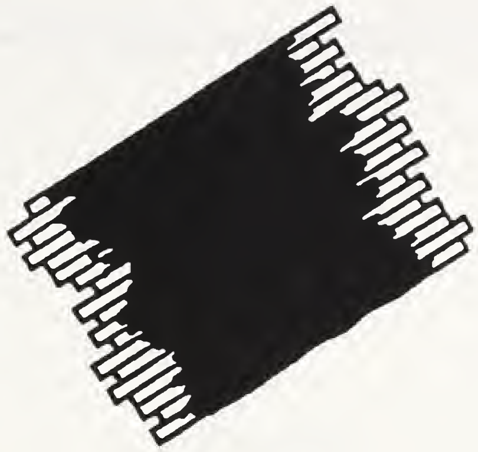
(for the above cases, the particle is $\geq$ 0.5 μm and the end of the asbestos fibers are possibly in the particle)

Counting bundles and clusters:

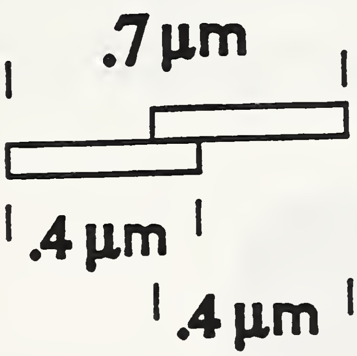
For a bundle or cluster to be countable, a fiber within the structure must have a ratio of 5:1 or greater and be longer than 0.5 µm. The overall ratio of dimensions of the bundle or cluster or its length are not relevant to determining a countable structure.



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



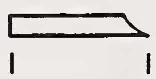
count as 0 structures
(cannot distinguish
fiber ≥ 0.5 s, ≥ 5:1 ratio)



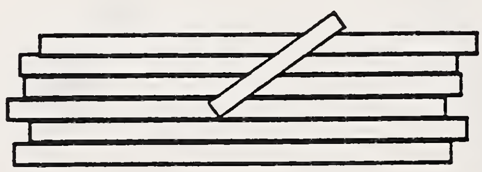
count as 0 structures
(does not have fiber ≥ 0.5 µm)

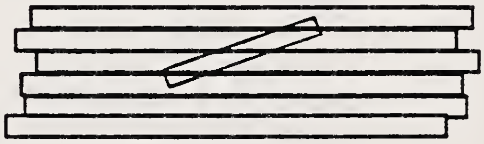
Special cases: Further definitions relating to AHERA counting rules

1. If the fiber to be measured has an irregular termination, measure the longest ending.



2. If a fiber or bundle intersects another fiber or bundle but does not protrude by 0.5 micrometers, it is still considered countable if it is ≥ 0.5 micrometers and has a length to width ratio ≥ 5:1. Examples are given below.





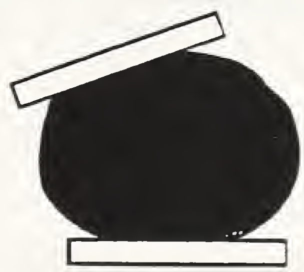
Both cases count as 2 structures

3. If three or more fibers intersect at the same place in a structure, count as three intersections and therefore as a cluster. See examples below.

Both arrangements count as one structure

4. 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). See examples below.

1 structure



2 structures

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

Form 6 - Recording form for Counting Protocol D

Lab code _______

Grid square _______

Field #	Structure sketch	Number of countable structures

Form 7 - Summary of counts for counting protocol D Lab code _______

Record in the table below the total number of countable structures found for each grid square.

Grid square	Total number of countable structures

Counting rules - Protocol E

Definitions:

Aspect ratio:
length to width ratio

Contiguous structure:
an agglomerate or series of touching particles (either asbestos or nonasbestos)

Fibril:
a single crystal of a regulated (asbestos) mineral that has an aspect ratio $\geq$ 5:1.
Note: there are no length restrictions

Occurrence of asbestos:
a single fibril or contiguous structure that contains at least one fibril.

INTEGER:
a function commonly used in computer programs in which the result of division of two numbers is truncated to the integer value. Note: the results are not rounded to the nearest integer. Examples:

INTEGER (1.1) = 1
INTEGER (1.9) = 1
INTEGER (8/3) = 2

Length unit:
a value defined as follows for these counting rules:

INTEGER (length of longest fibril in μm / 10 μm)

Long fibril unit:
a value defined as follows for these counting rules:

INTEGER ([# of fibrils $\geq$ 2 μm] / 5)

Short fibril unit:
a value defined as follows for these counting rules:

INTEGER ([# of fibrils < 2 μm] / 30)

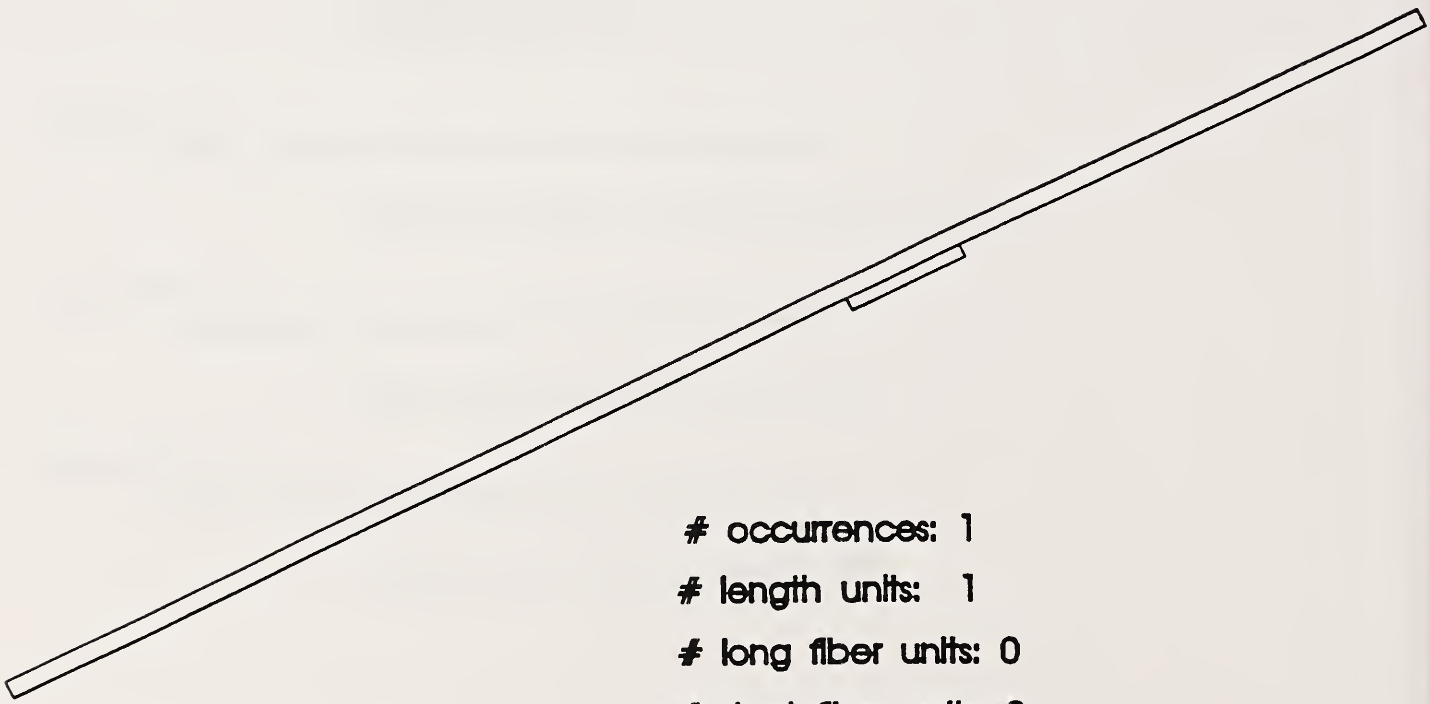
Counting rules:

1. Determine if the contiguous structure qualifies as an occurrence of asbestos.

2. Determine the number of length units, the number of long fibril units and the number of short fibril units for each occurrence of asbestos. Note: for a large number of occurrences, there will be not be five fibrils longer than 2 micrometers nor a fibril longer than 10 micrometers nor more than 30 short fibrils. Therefore, the number of length units and the number of short and long fibril units for the majority of occurrences will be 0.

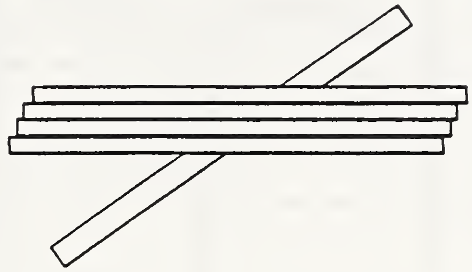
Examples of Counting Rules - Protocol E

occurrences: 1
length units: 0
long fiber units: 0
short fiber units: 0

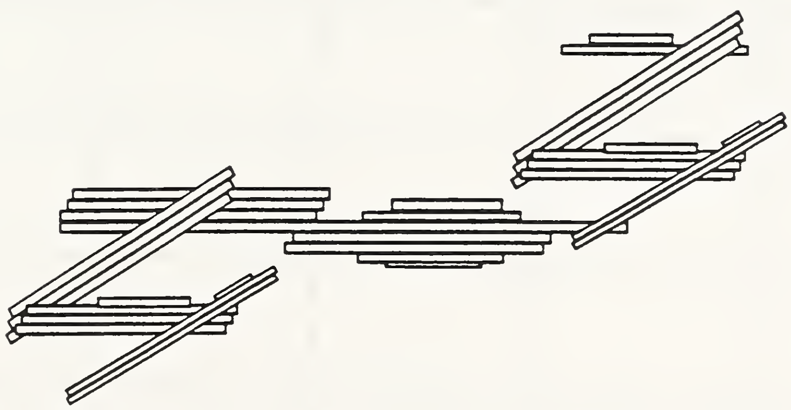


occurrences: 1
length units: 1
long fiber units: 0
short fiber units: 0

Protocol E - continued



occurrences: 1
length units: 0
long fiber units: 1
short fiber units: 0



occurrences: 1
length units: 0
long fiber units: 0
short fiber units: 1

Form 6 - Recording form for counting protocol E

Lab code _______

Grid square _______

Field of view	Sketch	# occurrences	# of length units	# of long fibril units	# of short fibril units

Form 7 - Summary of counts for Counting Protocol E Lab code ______

Grid square	# occurrences	# of length units	# of long fibril units	# of short fibril units

Form 8 Comment sheet

Lab code _____

List any ambiguities or difficulties with the set of counting rules given in this proficiency test. List any suggestions for improving the set of counting rules (attach more sheets if necessary).

Form 9 Counting rule sheet

Lab code ______

If you can derive a set of counting rules that you consider an improvement over the present rules, please describe them on this sheet (attach more sheets if necessary).

IV. Summary Report for PT90-2

The information presented in this report is a summary of the results and performance of laboratories on the proficiency test sent to laboratories in the airborne asbestos program in December of 1990 (designated as test 90-2). Discussion of results for both the diffraction pattern analysis and counting protocol analysis is given in the main portion of the report. A list of selected references for electron diffraction and crystallography is given in Appendix A. The results obtained by the laboratory receiving this report are given in Appendix B.

PART I - DIFFRACTION PATTERN ANALYSIS

Materials sent to laboratories

Laboratories were sent color copies of diffraction patterns from the following materials: 1) sputtered gold, 2) an "unknown", 3) chrysotile, and 4) tremolite-actinolite. Laboratories were asked to determine the camera constant from the sputtered gold pattern, d-spacings for labelled reflections on the "unknown" pattern, d-spacings and Miller indices for labelled reflections on the chrysotile and tremolite-actinolite patterns and the zone axis for the tremolite-actinolite pattern. One-hundred twenty-four laboratories returned results in this proficiency test.

Labelling of patterns

All of the laboratories were asked to determine d-spacings and Miller indices for the same reflections on the "unknown", the chrysotile and tremolite-actinolite diffraction patterns. However, the reflections were labelled differently depending upon which of the five counting protocols the laboratories received. The laboratories were divided into five groups, A-E, reflecting the protocol received by the laboratory (the group for the laboratory receiving this report is given next to the NVLAP lab code in Appendix B). In the discussions below and in Appendix B, results are reported in terms of the labelling scheme for laboratories in group A - those receiving protocol A. Keys are included in the sections that follow to indicate how the reflections have been renumbered for groups B-E. The keys need to be used only if a laboratory wants to compare results submitted in the proficiency test to the results discussed in this report.

Sputtered gold diffraction pattern

All laboratories received the same unlabelled sputtered gold diffraction pattern. The camera constant could have been reported as either a radius measurement (using a distance measurement from the central spot to a reflection) or as a diameter measurement (using the distance measurement between two equivalent reflections on either side of the central spot) (Hirsch et al., 1977).

The majority of laboratories reported the camera constant in terms of a radius measurement. One-hundred sixteen laboratories reported values ranging from 67.10 mm-Å to 70.60 mm-Å with a mean value of 69.29 mm-Å and standard deviation of 0.46 mm-Å. Four laboratories reported values in terms of a diameter measurement. Values were reported ranging from 137.80 mm-Å to 138.70 mm-Å with a mean value of 138.39 mm-Å and a standard deviation of 0.35 mm-Å.

For this proficiency test, all laboratories obtaining values in the ranges listed above for the radius and diameter measurements were marked acceptable. For this proficiency test only, those laboratories reporting values in units other than mm-Å were also marked correct if the converted values were within the acceptable ranges listed above. Those laboratories obtaining numerical values outside $\pm 10\%$ of the mean values were marked not acceptable.

For future determinations of camera constants on proficiency tests, we recommend that a minimum of three measurements at 45^{o} angles be made on the innermost ring. These measurements will allow detection of deviations of a ring from a circle. We further recommend that at least two measurements be made of the outer rings to make sure that there was not some gross error in measurement of the inner ring and to monitor for radial distortion.

"Unknown" diffraction pattern

The intent of this portion of the proficiency test was to determine if laboratories could analyze the d-spacings of diffraction patterns obtained from materials other than asbestos. The laboratory was asked to choose the reflections having the largest and second largest d-spacings and to report these d-spacings and their interplanar angle.

The reflections that should have been chosen by the laboratories as having the largest and second largest d-spacings are given in the following table:

	Groups A & D	Groups B & E	Group C
Largest d-spacing	4	2	4
Second largest d-spacing	3	1	5

There were three different labelling schemes for the diffraction patterns. The groups of patterns had slightly different enlargements when duplicated by the color copier. This caused a bimodal distribution of d-spacings for this material and therefore results obtained for d-spacing measurements are reported for each group of patterns.

A summary of the mean value and standard deviation for the largest d-spacing is given in the following table:

	Groups A & D	Groups B & E	Group C
mean value (Å)	3.43	3.42	3.45
st. dev. (Å)	0.06	0.06	0.06

A summary of the mean value and standard deviation for the second largest d-spacing is given in the following table:

	Groups A & D	Groups B & E	Group C
mean value (Å)	2.65	2.63	2.68
st. dev. (Å)	0.04	0.09	0.03

The angle between the two reflections should be the same for all groups. The range of accepted values obtained by the laboratories for the angle is 50-56^{o} and the mean value is 52.6^{o} with a standard deviation of 0.6^{o}.

Some laboratories interpreted the questions for this portion of the proficiency test incorrectly. The results for this section were therefore not used for evaluation of the laboratories.

<u>Chrysotile diffraction pattern</u>

The reflections were labelled on the diffraction patterns received by the five groups of laboratories as shown in the following table.

Numbering of reflections in this report	Numbering of reflections in proficiency test instructions				
	Group A	Group B	Group C	Group D	Group E
1	1	6	6	6	6
2	2	4	1	4	1
3	3	6	2	5	2
4	4	1	3	1	3
5	5	2	4	2	4
6	6	3	5	3	5

As an example of interpreting this table, if a laboratory is in Group B, the reflection labelled "3" in this report for chrysotile was labelled as reflection "5" on the diffraction pattern in the proficiency test instructions.

The d-spacings reported for chrysotile were analyzed as a group. Values for d-spacings that were within $\pm$ 10% of the mean value were considered to be correct. The values obtained by the laboratories are listed in the table that follows (using the reflection numbers for Group A):

Reflection	Mean (Å)	St. dev. (Å)
1	2.63	0.03
2	4.54	0.07
3	2.60	0.05
4	7.33	0.11
5	4.47	0.07
6	3.64	0.04

The unusual nature of chrysotile diffraction patterns lends itself to many correct (self-consistent) ways of indexing reflections. Four correct solutions for the Miller indices of chrysotile (using the reflection numbers for Group A) are given in the following table:

	1	2	3	4	5	6
Case 1	(200)	(110)	(130)	(002)	(020)	(004)
Case 2	(-200)	(-1-10)	(-1-30)	(00-2)	(0-20)	(00-4)
Case 3	(-200)	(-110)	(-130)	(002)	(020)	(004)
Case 4	(200)	(1-10)	(1-30)	(002)	(0-20)	(004)

Substitution of (201) for (200) was considered acceptable.

The electron diffraction pattern of chrysotile is unusual due to the curled structure of the mineral. Examples of indexed diffraction patterns of chrysotile are given in the references of the "chrysotile/serpentine minerals" section in Appendix A.

Tremolite-actinolite diffraction pattern

The reflections were labelled on the diffraction patterns received by the five groups of laboratories as shown in the following table.

Numbering of reflections in this report	Numbering of reflections in proficiency test instructions				
	Group A	Group B	Group C	Group D	Group E
1	1	4	1	1	1
2	2	5	2	2	2
3	3	1	6	6	3
4	4	2	3	3	4
5	5	3	4	4	5
6	6	6	5	5	6

The d-spacings reported for the tremolite-actinolite diffraction pattern were analyzed as a group. Values for d-spacings that were within $\pm 10\%$ of the mean value were considered to be correct. The values obtained by the laboratories are listed in the table that follows (using the reflection numbers of Group A):

Reflection	Mean (Å)	St. dev. (Å)
1	4.87	0.06
2	3.88	0.04
3	9.07	0.06
4	9.07	0.06
5	4.55	0.06
6	3.90	0.04

A common error in the determination of d-spacings for tremolite-actinolite resulted from the measurement of diffraction spots partially obscured by the central beam which occurred for reflections 3 and 4. Assuming there is a regular array of spots, it is more accurate to measure the distance over several spots and then to divide this distance by the appropriate number of diffraction spots measured. Because the d-spacing determination for reflections 3 and 4 involved the same measurement, only one error was assigned even if both d-spacings were incorrect.

The indexing of the tremolite-actinolite diffraction pattern was not used to judge the laboratory's proficiency for this round. The laboratory's results were, however, judged and the error assignments are given in parentheses in Appendix B. A description of how the answers were evaluated (and how they will be graded in the future) is given in the following text.

Four correct solutions for indexing the tremolite-actinolite diffraction pattern are as follows:

	1	2	3	4	5	6	ZONE
Case 1	(-111)	(-131)	(0-20)	(020)	(040)	(13-1)	[101]
Case 2	(11-1)	(13-1)	(0-20)	(020)	(040)	(-131)	[101]
Case 3	(-1-11)	(-1-31)	(020)	(0-20)	(0-40)	(1-3-1)	[101]
Case 4	(1-1-1)	(1-3-1)	(020)	(0-20)	(0-40)	(-1-31)	[101]

Another set of solutions was reported by several laboratories. For the cases below, d-spacings and interplanar angles are within measurement error of a correct solution, <u>however, extinction criteria are not fulfilled</u>:

	1	2	3	4	5	6	ZONE
Case 1	(011)	(031)	(0-20)	(020)	(040)	(03-1)	[100]
Case 2	(01-1)	(03-1)	(0-20)	(020)	(040)	(031)	[100]
Case 3	(0-11)	(0-31)	(020)	(0-20)	(0-40)	(0-3-1)	[100]
Case 4	(0-1-1)	(0-3-1)	(020)	(0-20)	(0-40)	(0-31)	[100]

Diffraction patterns indexed using incorrect extinction criteria are not considered acceptable. There were many other errors in the assignment of Miller indices for the tremolite-actinolite diffraction pattern. A listing of some of the errors and the manner in which they were graded are as follows:

1) Two identical Miller indices were given for different reflections on the same diffraction pattern:

   - one of the reflections was marked as an error.

2) Vector addition was performed incorrectly leading to inconsistent Miller indices:

   - the maximum number of indices consistent with any case of acceptable indexing of diffraction patterns was marked correct.

3) There was no correlation between the calculated d-spacing and the d-spacing corresponding to the Miller indices:

   - the Miller indices were marked as an error.

4) "Bars" (or "signs") on indices were deleted. This can lead to a major change in d-spacing. For example, for tremolite diffraction patterns:

Miller indices	d-spacing
(-111)	4.88 Å
(111)	3.97 Å
(13-1)	3.87 Å
(131)	3.37 Å

- these cases [(111) and (131)] were marked as errors.

5) A "bar zero" (-0) was given as one index:

- this was not marked as an error in Appendix B. However, it is not a correct "syntax" and it will not be accepted on future proficiency tests.

Some recommendations that may help in indexing reflections are as follows:

1) When indexing a diffraction pattern, it is, in general, better to index the reflections with the largest d-spacings first. The smaller d-spacings can have many d-spacings with a similar value and the chances of choosing an incorrect index are higher than for the larger d-spacings.

2) All reflections on a given diffraction pattern of a single crystal grain must correspond to the same zone. Therefore, calculating the cross product for any two reflections (see references on electron diffraction in Appendix A) should give the same zone axis. An exception to this is for curled structures such as chrysotile or halloysite for which reflections from more than one zone axis can be present on the same diffraction pattern.

3) When indexing a diffraction pattern, it is commonly necessary to calculate the d-spacing expected for a reflection. In addition, the indices should be verified by measuring interplanar angles and comparing them to calculated angles (note: interplanar angle here refers to the angle between reflections in reciprocal space). The equations for calculating d-spacings and interplanar angles for materials for which Miller indices are to be determined can be fairly complex, especially for monoclinic and triclinic minerals. The calculations can be done by writing programs for a programmable calculator or computer or by using a database-spreadsheet program that has a formula option.

Some concepts and items that a laboratory should become familiar with to aid in indexing include: the definition of Miller indices and how they relate to crystallographic planes in a crystal, the JCPDS file, equations for calculating d-spacings from Miller indices, equations for calculating the interplanar angle between two reflections using Miller indices, multiple diffraction, systematic extinctions, crystal classes, space groups, vector addition and calculation of a zone axis using a cross product of two Miller indices.

Discussion

Values for d-spacings within $\pm$ 10% of the mean value obtained by the laboratories were accepted for this proficiency test. In future proficiency tests, the range of acceptable values will be limited to less than $\pm$ 5%.

Laboratory personnel should note the areas in which they received errors in the listing of results for the diffraction portion of Appendix B and work to improve their understanding of material in these areas. The listing of references in Appendix A should provide some background information. The books in the "general electron diffraction" section of Appendix A have descriptions of the determination of the camera constant, d-spacings, Miller indices and zone axis. The International Tables provide information on systematic extinction criteria for the 230 space groups.

PART II - STRUCTURE COUNTING ANALYSIS

Materials sent to laboratories

All laboratories were sent identical color copies of prints of structures found on four grid squares and sketches of structures on a fifth grid square of a grid containing chrysotile asbestos. Laboratories were asked to determine values for the amount of asbestos present according to one of five counting protocols.

Description of counting protocols

The following list summarizes the essence of the five counting protocols - not all the details are included.

Protocol A: This protocol consisted of counting a single fibril (a single crystal of regulated asbestos mineral) or agglomerate of touching fibrils as one structure. Structures touching a grid bar counted as one-half a structure and touching two grid bars counted as one-third an asbestos structure.

Protocol B: This protocol consisted of counting the individual fibrils in an agglomerate of touching fibrils as one structure each. If more than six fibrils were identified, the agglomerate was assigned a value of six structures. Fibrils touching a grid bar counted as one-half of a structure and touching two grid bars counted as one-third of an asbestos structure.

Protocol C: This protocol consisted of the proposed draft method for the International Standards Organization. For this method, an area measurement is determined in addition to a fiber count.

Protocol D: This protocol consisted of the counting rules developed by EPA (Federal Register, 1987) with clarifications given in the first proficiency test and with further minor clarifications.

Protocol E: This protocol consisted of four parts as follows:

1) Determination of the number of "occurrences" as described for Protocol A (without the grid bar ruling).

2) Determination of the number of "length units" [or the (length of the longest fibril)/10μm].

3) Determination of the number of "long fibril units" [or the (number of fibrils $\geq$ 2 μm)/5].

4) Determination of the number of "short fibril units" [or the (number of fibrils < 2 μm)/30].

Summary of results

The preliminary results for the structure counts obtained by the laboratories on the five grid squares are given in the following tables:

Protocol	Mean structure count	Standard deviation	Coefficient of variation
A	37.9	1.6*	0.04
B	137.3	31.0	0.23
C	69.2	8.5	0.12
D	63.6	12.1	0.19
E	39.9	1.9	0.05

* The reported standard deviation for protocol A does not include an outlier result obtained for one laboratory. The inclusion of the results for this laboratory gives a standard deviation of 3.8.

Other values obtained by the laboratories include the following:

Protocol	Type of determination	Value	Standard deviation
C	area (μm^2)	8.7	10.0
E	length units	3.8	1.0
E	long fiber units	5.7	2.3
E	short fiber units	2.3	2.2

Discussion

The preliminary analysis of data from the counting protocols indicates that the highest precision is given by the simple fibril counting rules used for Protocol A and for the first part of Protocol E. The fibril counts for Protocol B, C and D have a fairly high imprecision likely indicating problems with the applications of these methods. The determination of length units for Protocol E showed a higher precision than obtained for the short and long fiber units.

The preliminary data indicate that the simple fibril counting rules used for Protocol A and for the first part of Protocol E should be incorporated into a future counting method. Further discussion and testing will be conducted prior to adoption of a new counting protocol.

PART III - DISCUSSION OF GRADING OF THE PROFICIENCY TEST

The laboratories could obtain a maximum of eighteen errors on the diffraction portion of the proficiency test: the camera constant, the d-spacings and Miller indices for chrysotile and the d-spacings for tremolite-actinolite. Six errors were given if a laboratory did not satisfactorily complete the counting portion of the proficiency test. A laboratory passed this proficiency test if it accumulated less than two errors. Nineteen laboratories did not pass this proficiency test.

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Chrysotile/Serpentine Minerals:

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