SAMPLING AND EVALUATING AIRBORNE ASBESTOS DUST

(582)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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National Institute for Occupational Safety and Health
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Authors: S. G. Bayer.

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Abstract: Contents: Agenda; Course description; Pretest; METHOD: 7400; Asbestos analysis; Overloading and electrostatic effects; Pump calibration; Asbestos sampling - rules for good guessing; Introduction to statistics; Familiarization with the microscope; Setting up the microscope - slide show; Graticule calibration; Walton-Beckett-images; Filter mounting; Quality-control testing of asbestos counts; METHOD: 7402; METHOD: 7403; Count sheets.

CONTENTS

NIOSH Course #582

Sampling & Evaluating Airborne Asbestos Dust

Presented by:

Centers for Disease Control

National Institute for Occupational Safety and Health

Division of Training & Manpower Development

Direct Training Branch

4676 Columbia Parkway

Cincinnati, Ohio 45226

Division Director: Thomas Purcell Ph. D.

Branch Chief: Michael Colligan, Ph. D.

Course Director: Stephen G. Bayer, Sr.

General Branch Telephone #: (513) 533-8225

Occasional Guest Lecturers

Dr. Paul Baron, (513) 841-4266
Division of Physical Sciences & Engineering

Eugene Moss (513) 841-4374
Division of Surveillance, Hazard Evaluations, & Field Studies

The above address applies to <u>all</u> of Cincinnati's operations

NIOSH Course #582

Sampling and Evaluating Airborne Asbestos Dust

Course Description, Requirements, and Recommendations

by Stephen G. Bayer, Sr.

March, 1988

The following course information can help students decide whether the NIOSH 582 course is appropriate for their needs, determine whether they should prepare (in advance) for the course, and can provide comparative information for individuals to use when seeking an "equivalent" course.

Since course attendance is mandated by law, the course is geared to the needs of the majority. Most students have at least four years of college and some experience with sampling or analysis. Thus, the course's instructional level is adjusted to provide a review of important aspects of the method and, more importantly, to train and educate such students to a higher level of professional technique and knowledge.

AGENDA

8:00 - 4:30 DAILY:

Monday: Sampling

Tuesday: Statistics and counting introduction

Wednesday: Microscopy

Thursday: Counting, Filter Mounting, Computer Quizzes

Friday: Continuation (AM) and Examination (PM)

8:00 - 8:30 Tuesday - Friday: Homework review

There are no closing formalities in the course. When a student's exam is graded (first finished, first graded) the student receives the course certificate and grade document and the course is over.

With rare exception (students preferring extended examination time), 4:00 PM is a reasonable expectation for departure.

COURSE ACTIVITIES

During the course, students:

Calibrate personal-samplers

Prepare slide mounts of filters

Adjust and calibrate microscopes

Count filters

Participate in mathematical exercises associated with sampling, statistics (compliance testing and quality control), microscope calibration, and concentration determinations.

WHAT THE COURSE COVERS

NIOSH method 7400! The course explains the equipment, procedures, and calculations that are required and recommended by the method. Some similarities and differences with other methods are discussed.

Instruction is accomplished by lectures, hands-on practice sessions, homework, and computer techniques.

Copies of the public-domain computer software (Apple II+ only) used in the course are available, on request, to students.

Principal topic areas include sampling, filter mounting, microscopy, calculations, and statistical procedures.

A course manual is provided to each student. Content includes official NIOSH publications, personal lecture notes, and training-oriented data forms.

Students may be required to share microscopical equipment with others (two to a microscope). To extend the number of students, some students may be required to supply their own microscopes and accessories.

TOPICS NOT COVERED

The course emphasis and purpose is to provide training and education on phase-contrast types of fiber evaluations. The course is not intended to cover:

Details of regulations

Bulk identification

Control measures

Work practices

MEASURING SKILLS

Pump calibration data is compared to values (generated by previous classes) for the same or similar equipment.

Mounted slides are submitted to the course director for evaluation and advise.

Fiber counts, from reference slides, are entered into a computer data base and are assigned illustrative grades based on proximity to the mean.

Homework is reviewed in class each morning, following instruction. The homework is not collected and completion could be considered voluntary. However, conscientious completion of the homework will improve the final exam grade.

Technical knowledge is measured using a written comprehensive examination having 100 questions. The current exam was created 8/87 and is occasionally revised for accuracy and clarity. The exam can cover anything from the method. A few questions test relevant knowledge outside the specific training aspects of the course.

For the exam, students may prepare an 8.5" X 11" page (both sides) of handwritten notes and should have a scientific type of calculator. The examination is designed to be very challenging at the expert level.

EXAM PERFORMANCE SUMMARY

Forty three industrial hygienists averaged 80%. Of those, five were ABIH Certified Industrial Hygienists who averaged 92% and ranged between 80% to 98%. Three students indicated that they were ABIH Certified Industrial Hygiene Technicians, averaging 73%, and ranging between 58% to 95%.

Eight students indicated participation in the AIHA asbestos-analysis registry. The average was 72%, ranging between 41% to 88%.

Those with no college degree averaged 62% and ranged between 25% to 96% Eighteen had associate degrees and , averaged 65% and ranged between 19% to 91%. Eighty two had bachelor degrees, averaging 81% and ranged between 33% to 97%. Thirteen had a master's degree, averaging 84% and ranging between 55% and 98%. Five having doctorate degrees averaged 86% and ranged between 67% to 99%.

VERIFICATION OF ATTENDANCE

To verify that the course has been "taken" (i.e. completed), students receive an official NIOSH/DTMD certificate of attendance.

An informal grade document is also issued with the certificate. The document is signed by the course director and shows the grade for the final written examination. The document also summarizes the examination performance (sorted by academic background) of previous students.

The document is to be used for personal purposes, such as attachment to the course certificate for personnel records (when needed). In this fashion, one can demonstrate personal achievement by comparison to one's peers. NIOSH does not maintain a file of student's grades (by name).

To complete the course, one must be in attendance, participate in course activities, and complete the final examination. Effective 10/87 (with the exception of students from countries other than the United States of America), anyone leaving before the completion and grading of their respective final examination, WILL NOT receive a course certificate of completion.

RECOMMENDED PREREQUISITES

The NIOSH 582 course is not intended to be a replacement for on-the-job training, in-house training, and academic accomplishments. Student background profiles range from high-school education to doctoral degrees and span a wide range of experience levels. The less experience and scientific education one has, the more difficult the course.

Those not familiar with the method should study the attached copy of the method prior to attendance.

Students should also obtain a scientific calculator and practice operations and functions such as squaring numbers, square roots, and entering and recalling numbers from the memory. Statistical functions may also be helpful. Know how to use the calculator before the course.

COURSE HISTORY

"The Method Used by the U.S. Public Health Service for Enumeration of Asbestos Fibers on Membrane Filters", by George H. Edwards and Jeremiah R. Lynch, was published in Annals of Occupational Hygiene, Volume II, pp 1-6, Pergammon Press 1968. Printed in Great Britain

Effective July, 1972, OSHA sets a 5 fib/cc Permissible Exposure Limit , a 10 fib/cc Ceiling Limit, and requires the "membrane filter method" to be used for monitoring.

First NIOSH "asbestos course" presented 10/31/72- title: "Sampling and Evaluating Airborne Asbestos Dust". The 2.5 day course covered the "membrane filter method." Designed and directed by Stephen G. Bayer, Sr. The numbering scheme (i.e. Course #582) began ca. 1975.

Effective July, 1976, the OSHA PEL was lowered to 2 fib/cc. The Ceiling Limit remained at 10 fib/cc.

NIOSH Physical and Chemical Analytical Method 239 (P&CAM 239) was issued 3/30/77. Soon thereafter, it was published in the NIOSH Manual of Analytical Methods, Second Edition, Volume 1, April 1977.

In February, 1979, details on P&CAM 239 were explained in a detailed technical report entitled USPHS/NIOSH Membrane Filter Method for Evaluating Airborne Asbestos, Publication #79-127, by Nelson A. Leidel, Stephen G. Bayer, Ralph D. Zumwalde, and Kenneth A. Busch. DHEW (NIOSH).

Method 7400 was issued 2/15/84 as replacement for P&CAM 239. Method 7400 was written by James W. Carter, David G. Taylor Ph.D. CIH, and Paul A. Baron, Ph.D. (NIOSH/DPSE). Revision #2 was issued 5/15/86. A later printing of revision #2 is dated 8/15/87 and, as of 3/88, is the latest revision.

In 1987, the OSHA PEL was lowered to 0.2 fib/mL, an Action Limit of 0.1 fib/mL was established, and "All individuals performing asbestos ... analysis must have taken the NIOSH course ... or an equivalent ..."

Considering the course significance (relative to the OSHA regulations), the course was extended in October, 1987, to five full days, providing additional time for counting and for a thorough final exam.

NAME: _____

NIOSH Course #582

Homework

Practice Examination

3/88

Complete, on a daily basis, appropriate sections of this homework examination. Daily assignments will be reviewed on the following morning (8:00 - 8:30).

Nightly Assignments:

Monday:	Questions # 1 - #36
Tuesday:	#37 - #60
Wednesday:	#61 - #82
Thursday:	#83 - #99

NOTES FOR FINAL EXAMINATION:

Students are permitted a scientific calculator and one 8.5" X 11.5" sheet (both sides) of handwritten notes to use as a resource.

All counting-related questions will be based upon the "A" counting rules in method #7400.

Sampling and Standards

#1 OSHA PEL is _____ fibers/mL.

#2 OSHA Action Limit is _____ fibers/mL.

#3 NIOSH recommended 8 hr TWA is _____ fibers/mL.

#4 TLV for chrysotile is _____ fibers/cc.

#5 The Limit Of Detection is _____ fibers/mm^2.

#6 The Limit Of Quantitation is _____ fibers/mm^2.

#7 The specified counting range of the method is _____ to _____ fibers/mm^2.

#8 The range of pore sizes permitted by Method 7400 is from _____ to _____ um (micrometers).

#9 The filter composition is _____.

#10 The minimum flow rate (Method 7400) for a 25 mm filter is _____ L/min.

#11 The maximum flow rate (Method 7400) is _____ L/min.

#12 In the OSHA reference method (ORM), the maximum flow rate is limited to _____ L/min.

#13 The minimum flow rate for a 37 mm filter is _____ L/min.

#14 A calibration standard having a fixed volume that can be determined by direct measurement, independent of the gas or air involved in the calibration process, is called a (an) _____ standard.

#15 An airflow calibration standard, usually used as a routine flow metering or indicating device. is a (an) _____ standard.

#16 An airflow-calibration device which is nearly as accurate and precise as a primary, but cannot meet the definition and must be periodically calibrated is a (an) _____ standard.

Classify by type of calibration standard

#17 Rotameter: _____

#18 Solid state all electronic flow meter: _____

#19 Electronic soap-film meter: _____

#20 Dry Gas Meter: _____

#21 Wet Test Meter: _____

#22 Water-filled Spirometer _____

#23 When one measures several time trials at a given rotameter number, averages the number of seconds, and tests the average against a ± 5% criterion, one is checking the _____ (reproducibility) of the calibration.

#24 The _____ (true value) of the calibration is dependant on the type of calibration standard.

#25 When a rotameter is located between the pump mechanism and the filter, the filter resistance affects the _____ of the rotameter.

#26 If one were to measure a volume of 750 mL, drawn over an average time of 21.4 seconds, the flow rate would be _____ (nearest 0.1 L/min).

#27 Determine the time-weighted average:

	fib/mL	minutes
#1	0.31	80
#2	0.17	114
#3	0.14	98
#4	0.12	127

TWA = _____ fib/mL

#28 The typical sampling area of a 25 mm filter is _____ mm^2.

#29 The typical sampling area of a 37 mm filter is _____ mm^2.

#30 The average result from lab blanks cannot be equal to or greater than a total count of _____ fibers.

#31 If any field blank has more than _____ fibers in 100 fields, then one should report possible contamination of the samples.

#32 Calibrated flow rate 3.0 L/min
Pressure when calibrating..... 753 mm Hg
Pressure when sampling..... 748 mm Hg
Temperature when calibrating... 22' C
Temperature when sampling..... -12' C
Flow rate adjusted to calibration: _____ (nearest 0.01 L/min)

#33 Average count of sample..... 0.83 fib/fld

Average count of blanks..... 0.045 fib/fld

Effective filtration area.... 381. mm^2

Field area..... 0.00770 mm^2/fld

Flow rate..... 2.5 L/min

Sample time..... 316. min

Conversion factor..... 1000 mL/L

Concentration _____ (nearest 0.01 fib/mL)

34 Average count _____ (nearest 0.1 fib/fld)

Filter area..... 385.

Field area..... 0.00785

Flow rate..... 1.5

Sample time..... 210.

Concentration... 0.2

#35 Flow rate _____ liters/minute (nearest 0.1)

Concentration... 0.3

Filter area..... 385.

Average count... 0.8

Field area..... 0.00785

Sample time..... 178.

#36 Sample time _____ minutes (nearest whole minute)

Concentration... 0.05

Average count... 1.0

Filter area..... 385.

Flow rate..... 10.0

Field area..... 0.00785

STATISTICS

fib/mm^2

745

296

276

296

470

653

458

392

463

333

#37 Average (u) _____ #38 Standard Deviation (S) _____

#39 Relative Standard Deviation (S_r or CV) _____

#40 2 Sided 95% Confidence Interval _____ - _____

#41 1 Sided Upper 95% Confidence Limit _____

#42 1 Sided Lower 95% Confidence Limit _____

#43 The _____ is the most commonly seen value.

#44 The _____ is a value that represents a midpoint in the data. In a normal dist., one could say that half the data is equal to or less than this value.

#45 A _____ bias tends to shift the accuracy of measurement.

#46 _____ error tends to cause the exact value to be uncertain.

#47 CV X MEAN = _____.

#48 Mean $\pm$ (1.965 X S) calculates:

A The compliance status for a sample

B Statistical Limit of Quantitation

C Upper 95% Confidence Limit (1 sided)

D 1 Sided Lower 95% Confidence Limit

E 95% Confidence Limits (2 sided upper and lower)

#49 Mean - (1.645 X S) calculates:

A The degree of compliance for a sample

B Warning Limits

C Upper 95% Confidence Limit (1 sided)

D 1 Sided Lower 95% Confidence Limit

E 95% Confidence Limits (2 sided upper and lower)

#50 The relative standard deviation (S_r or RSD) is also known as Coefficient of Variation (CV). It is calculated by dividing the standard deviation by the _____.

#51 In a quality-control program, there are values used as indicators for results which are too far away from the average. These indicators are usually termed _____ limits.

#52 There are also two indicators, used as flags, for data which tends to be higher or lower than most other results. These indicators are termed _____ limits.

#53 When the mean, the median, and the mode are all the same value (or reasonably close), one considers the distribution to be _____ distributed.

COUNTING

#54 In order for a fiber to be counted as 1, it must be entirely within the field and be at least _____ times as long as the maximum thickness.

#55 A eligible fiber crossing the field boundary more than one time is counted as _____ (1, 1/2, or 0).

#56 An eligible fiber crossing the field boundary only one time is counted as _____ (1, 1/2, or 0).

#57 To be eligible for counting, a fiber must be longer than _____ um (micrometers).

#58 When more than 1/6 of a counting field is obliterated by a mass of material (air bubble, particle, etc.), what procedure is followed?

#59 Which set of counting rules in Method 7400 were retained from P&CAM 239 and are specified in the OSHA Reference Method (ORM)? _____ Rules

#60 On already-light samples, as the number of fibers counted continues to decrease, one expects the S_r to _____.

Microscopy

#61 The lens assemblies into which one looks when using a microscope are called _____.

#62 Inside one lens assembly is a small glass plate on which the counting grid is found. This glass disc is called a (an) _____.

#63 The 40X lens assembly which focuses on the sample is called a (an) _____.

#64 Inside this lens assembly there appears to be a dark ring called a _____.

#65 The large lens assembly found just underneath the slide or stage is called a (an) _____.

#66 Inside this assembly, there is a plate which passes a ring of light. This plate is called the _____.

#67 The permissible range of numerical aperture is _____ - _____.

#68 The microscope must be _____ phase contrast.

#69 Centering of the phase rings is best observed by using a centering _____.

#70 The microscope is calibrated by using a very small ruler deposited on a microscope slide. The slide is called a (an) _____ _____.

#71 There is a special slide used to determine the phase shift detection limit (i.e. resolution/contrast capability of the microscope). The <u>exact</u> description of this slide is _____.

#72 _____ illumination is preferred, where available.

#73 True or False- The three basic steps for adjusting the microscope, listed in proper order, are:

1) Focus on the sample

2) Adjust the phase rings TRUE OR FALSE

3) Adjust the field iris.

#74 An adjustable illuminator opening, which defines the diameter of the illumination beam, is called the _____ _____.

#75 In the condenser, there is another adjustable opening, <u>not</u> used for phase-contrast illumination. This other opening is called the _____ _____.

#76 The proper graticule (for the A counting rules) is a Walton-Beckett G- _____.

#77 When using the resolution/contrast test slide, one must be able to see the fourth or fifth set of lines PARTIALLY. The finest set of lines which must be <u>completely</u> visible is set # _____.

#78 The overall magnification, at counting status, must be approximately _____X.

#79 The cover glass should be considered a high-quality parallel lens. There is a specific thickness rating of #1 1/2 needed to maintain spherical aberration at a minimum. The thickness in millimeters is _____.

#80 When a graticule is ordered, three items must be specified; the field diameter, the type (i.e. G-24), and the _____.

#81 If the diameter of the graticule is 0.099 mm, what is the field area is _____ mm^2.

#82 What counting field diameter would be ordered for a microscope if an actual graticule dimension of 4.32 mm was magnified to a dimension of 0.152 mm? _____ mm

Compliance Testing Statistics

#83 The Null hypothesis requires that the _____ (on the right side of the 1 sided 95% statistical equation) replace the mean.

#84 For an employer to be 95% sure compliance has been achieved, both the upper 95% 1 sided confidence limit and the measurement must be _____ the standard.

#85 For NON-COMPLIANCE to be established, at 95% probability, both the measurement and the _____ must be higher than the standard.

#86 If the measured concentration is 0.04 fib/mL and the coefficient of variation is 0.21, determine the compliance status for a standard of 0.1 fib/mL.

A In Violation NOTE: EMPLOYER'S VIEWPOINT

B Possible Violation

C In Compliance

#87 If the measured concentration is 0.28 and the S_r is 0.30, determine at (P = 0.95) the compliance status for a standard of 0.20 fib/mL.

A In Violation NOTE: REGULATORY AGENCY'S VIEWPOINT

B Possible Violation

C In Compliance

#88 For 90 fibers counted, the Poisson distribution would predict a relative standard deviation of _____?

#89 For 90 fibers counted, Ogden's equation predicts a S_r of _____.

#90 Method 7400, in the section on interlaboratory variability states that, in the absence of other information, use an S_r value of _____.

#91 Two samples were taken at a given operation and on a given individual. The fiber densities were quite similar expecting a S_r of 0.20. The measured concentrations were 0.13 and 0.15 fib/mL. Each sample was taken for 3 hours and the remaining time of the 8 hour day is expected to be quite similar to the sampled period. At 95% probability:

Assuming the OSHA PEL and an employer's perspective, determine the compliance status.

Filter Mounting

#92 For the non-permanent mounting procedure (used in P&CAM 239, described in method 7400 as an optional field-mounting procedure), list the common names for the two liquids.

_____

and

_____

#93 These liquids are added to one another in what ratio by volume? _____

#94 How many grams of clean, white plain filter material is added to the solution to create, at a minimum viscosity, 15 mL?

_____ grams (nearest 0.01)

#95 What is a "rule of thumb" when filter mounting?

__AVOID__ _____!

#96 The permanent slide-mounting procedure requires softening the filter in _____ vapor.

#97 The partially-cleared filter is rendered "clear" by adding (for 1/4 of a 25 mm filter) about 3.5 uL of a liquid commonly called _____?

#98 The proper coverglass thickness is 0.17 mm or #_____.

#99 The filter is placed on the slide
_____ _____ _____. (3 words)

FORMULA: various

M.W.: various

FIBERS

METHOD: 7400
ISSUED: 2/15/84
REVISION #2: 8/15/87

OSHA: 0.2 asbestos fibers (> 5 µm long)/mL [1]
NIOSH: 0.1 asbestos f/mL [1]; 3 glass fibers (>10 µm x <3.5 µm)/mL [3]
ACGIH: 0.2 crocidolite; 0.5 amosite; 2 chrysotile and other asbestos, f/mL

PROPERTIES: solid, fibrous

SYNONYMS: actinolite asbestos [CAS #13768-00-8], grunerite asbestos (amosite) [CAS #12172-73-5], anthophyllite asbestos [CAS #17068-78-9], chrysotile asbestos [CAS #12001-29-5], crocidolite asbestos [CAS #12001-28-4], tremolite asbestos [CAS #14567-73-8]; fibrous glass.

SAMPLING

SAMPLER: FILTER
(0.8- to 1.2- µm cellulose ester membrane, 25-mm diameter; conductive cowl on cassette)

FLOW RATE*: 0.5 to 16 L/min (see step 4)

VOL-MIN*: 400 L @ 0.1 fiber/mL (see step 4)
-MAX*: (see step 4)
*Adjust for 100 to 1300 fibers/mm² (step 4)

SHIPMENT: routine (securely packed to reduce shock)

SAMPLE STABILITY: stable

FIELD BLANKS: 10% (≥2) of samples

ACCURACY

RANGE STUDIED: 80 to 100 fibers counted

BIAS: see EVALUATION OF METHOD

OVERALL PRECISION (s_r): 0.115 to 0.13 (A Rules) [3]

MEASUREMENT

TECHNIQUE: LIGHT MICROSCOPY, PHASE CONTRAST

ANALYTE: fibers (manual count)

SAMPLE PREPARATION: acetone/triacetin "hot block" method [5]

COUNTING RULES: Set A (required by OSHA; [1,4]) or Set B (modified CRS [6])

EQUIPMENT: 1. positive phase-contrast microscope
2. Walton-Beckett graticule (100-µm field of view): A Rules use Type G-22; B Rules use Type G-24
3. phase-shift test slide (HSE/NPL)

CALIBRATION: HSE/NPL test slide

RANGE: 100 to 1300 fibers/mm² filter area

ESTIMATED LOD: 7 fibers/mm² filter area

PRECISION: 0.10 to 0.12 (A Rules) [3]
(see Evaluation of Method:B)

APPLICABILITY: The method gives an index of airborne fibers in workplace atmospheres. Phase contrast microscopy will not differentiate between asbestos and other fibers; use this method in conjunction with electron microscopy (e.g., Method 7402) for positive identification. Fibers < ca. 0.25 µm diameter will not be detected by this method [7].

INTERFERENCES: Any other airborne fiber may interfere since all particles meeting the counting criteria are counted. Chain-like particles may appear fibrous. High levels of non-fibrous dust particles may obscure fibers in the field of view and increase the detection limit.

OTHER METHODS: This method introduces changes for improved sensitivity and reproducibility. It replaces P&CAM 239 [4,8] and Method 7400, Revision #1 (dated 5/15/85).

REAGENTS:

1. Acetone.*
2. Triacetin (glycerol triacetate), reagent grade.

*See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: field monitor, 25-mm, three-piece cassette with ca. 50-mm electrically-conductive extension cowl and cellulose ester filter, 0.8- to 1.2-µm pore size, and backup pad.
   NOTE 1: Analyze representative filters for fiber background before use. Discard the filter lot if mean is ≥ 5 fibers per 100 graticule fields. These are defined as laboratory blanks.
   NOTE 2: Use an electrically-conductive extension cowl to reduce electrostatic effects. Ground the cowl when possible during sampling.
2. Personal sampling pump, 0.5 to 16 L/min (see step 4 for flow rate), with flexible connecting tubing.
3. Microscope, positive phase contrast, with green or blue filter, 8 to 10X eyepiece, and 40 to 45X phase objective (total magnification ca. 400X); numerical aperture = 0.65 to 0.75.
4. Slides, glass, frosted-end, pre-cleaned, 25 x 75 mm.
5. Cover slips, 22 x 22 mm, No. 1-1/2, unless otherwise specified by microscope manufacturer.
6. Lacquer or nail polish.
7. Knife, #10 surgical steel, curved blade.
8. Tweezers.
9. Heated aluminum block for clearing filters on glass slides (see ref. [5] for instructions on manufacture).
10. Micropipets, 5-µL and 100- to 500-µL.
11. Graticule, Walton-Beckett type with 100-µm diameter circular field (area = 0.00785 mm²)at the specimen plane (Type G-22 for A Rules; Type G-24 for B Rules). Available from PTR Optics Ltd., 145 Newton Street, Waltham, MA 02154 [phone (617) 891-6000] and McCrone Accessories and Components, 850 Pasquinelli Drive, Westmont, IL 60559 [phone (312) 887-7100].
    NOTE: The graticule is custom-made for each microscope. Specify disc diameter needed to fit exactly the ocular of the microscope and the diameter (mm) of the circular counting area (see APPENDIX A).
12. HSE/NPL phase contrast test slide, Mark II. Available from PTR Optics Ltd. (address above).
13. Telescope, ocular phase-ring centering.
14. Stage micrometer (0.01-mm divisions).
15. Wire, multi-stranded, 22-gauge.

SPECIAL PRECAUTIONS: Acetone is extremely flammable. Take precautions not to ignite it. Heating of acetone in volumes greater than 1 mL must be done in a ventilated laboratory fume hood using a flameless, spark-free heat source.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.

2. For personal sampling, fasten sampler to the worker's lapel near the worker's mouth. Remove top cover from cowl extension (open face) and orient face down. Wrap joint between cowl and monitor body with shrink tape to prevent air leaks.
   NOTE: If possible, ground the cassette to remove any surface charge, using a wire held in contact (e.g., with a hose clamp) with the conductive cowl and a non-electrical metal fixture, or a cold-water pipe.
3. Submit at least two field blanks (or 10% of the total samples, whichever is greater) for each set of samples. Remove top covers from the field blank cassettes and store top covers and cassettes in a clean area (bag or box) with the top covers from the sampling cassettes during the sampling period. Replace the top covers in the cassettes after sampling.
4. Sample at 0.5 L/min or greater [9]. Adjust sampling flow rate, Q (L/min), and time, t (min), to produce a fiber density, E, of 100 to 1300 fibers/mm^2 ($3.85 \cdot 10^4$ to $5 \cdot 10^5$ fibers per 25-mm filter with effective collection area A_c= 385 mm^2) for optimum accuracy. These variables are related to the action level (one-half the current standard), L (fibers/mL), of the fibrous aerosol being sampled by:

$$t = \frac{(A_c)(E)}{(Q)(L)10^3}.$$

   NOTE 1: The purpose of adjusting sampling times is to obtain optimum fiber loading on the filter. A sampling rate of 1 to 4 L/min for 8 hrs is appropriate in non-dusty atmospheres containing ca. 0.1 fiber/mL. Dusty atmospheres require smaller sample volumes ($\leq$400 L) to obtain countable samples. In such cases take short, consecutive samples and average the results over the total collection time. For documenting episodic exposures, use high flow rates (7 to 16 L/min) over shorter sampling times. In relatively clean atmospheres, where targeted fiber concentrations are much less than 0.1 fiber/mL, use larger sample volumes (3000 to 10000 L) to achieve quantifiable loadings. Take care, however, not to overload the filter with background dust. If $\geq$ 50% of the filter surface is covered with particles, the filter may be too overloaded to count and will bias the measured fiber concentration.
   NOTE 2: OSHA regulations specify a maximum sampling rate of 2.5 L/min [1].
5. At the end of sampling, replace top cover and small end caps.
6. Ship samples with conductive cowl attached in a rigid container with packing material to prevent jostling or damage.
   NOTE: Do not use untreated polystyrene foam in shipping container because electrostatic forces may cause fiber loss from sample filter.

SAMPLE PREPARATION:

NOTE: The object is to produce samples with a smooth (non-grainy) background in a medium with refractive index $\leq$ 1.46. This method collapses the filter for easier focusing and produces permanent mounts which are useful for quality control and interlaboratory comparison. The aluminum "hot block" technique may be used outside the laboratory [5]. Other mounting techniques meeting the above criteria may also be used (e.g., the laboratory fume hood procedure for generating acetone vapor as described in Method 7400 - revision of 5/15/85, or the non-permanent field mounting technique used in P&CAM 239 [2,4,8,22]). A videotape of the mounting procedure is available from the NIOSH Publication Office [20].

7. Ensure that the glass slides and cover slips are free of dust and fibers.
8. Adjust the rheostat to heat the "hot block" to ca. 70 °C [5].
   NOTE: If the "hot block" is not used in a fume hood, it must rest on a ceramic plate and be isolated from any surface susceptible to heat damage.

9. Mount a wedge cut from the sample filter on a clean glass slide.
   a. Cut wedges of ca. 25% of the filter area with a curved-blade steel surgical knife using a rocking motion to prevent tearing. Place wedge, dust side up, on slide.
      NOTE: Static electricity will usually keep the wedge on the slide.
   b. Insert slide with wedge into the receiving slot at base of "hot block". Place tip of a micropipet containing ca. 250 µL acetone into the inlet port of the PTFE cap on top of the "hot block". Inject the acetone into the vaporization chamber with a slow, steady pressure on the plunger button while holding pipet firmly in place. After waiting 3 to 5 sec for the filter to clear, remove pipet and slide from their ports.
      CAUTION: Although the volume of acetone used is small, use safety precautions. Work in a well-ventilated area (e.g., laboratory fume hood). Take care not to ignite the acetone. Continuous, frequent use of this device in an unventilated space may produce explosive acetone vapor concentrations.
   c. Using the 5-µL micropipet, immediately place 3.0 to 3.5 µL triacetin on the wedge. Gently lower a clean cover slip onto the wedge at a slight angle to reduce bubble formation.
      NOTE: If too many bubbles form or the amount of triacetin is insufficient, the cover slip may become detached within a few hours. If excessive triacetin remains at the edge of the filter under the cover slip, fiber migration may occur.
   d. Glue the edges of the cover slip to the slide using lacquer or nail polish [10] Counting may proceed immediately after clearing and mounting are completed.
      NOTE: If clearing is slow, warm the slide on a hotplate (surface temperature 50 °C) for up to 15 min to hasten clearing. Heat carefully to prevent gas bubble formation.

CALIBRATION AND QUALITY CONTROL:

10. Microscope adjustments. Follow the manufacturers instructions. At least once daily use the telescope ocular supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric. With each microscope, keep a logbook in which to record the dates of microscope cleanings, adjustments, and calibrations.
   a. Each time a sample is examined, do the following:
      (1) Adjust the light source for even illumination across the field of view at the condenser iris. With some microscopes, the illumination may have to be set up with bright field optics rather than phase contract optics.
          NOTE: Use Köhler illumination if available.
      (2) Focus on the particulate material to be examined.
      (3) Make sure that the field iris is in focus, centered on the sample, and open only enough to fully illuminate the field of view.
   b. Check the phase-shift detection limit of the microscope periodically for each analyst/microscope combination:
      (1) Center the HSE/NPL phase-contrast test slide under the phase objective.
      (2) Bring the blocks of grooved lines into focus in the graticule area.
          NOTE: The slide contains seven blocks of grooves (ca. 20 grooves per block) in descending order of visibility. For asbestos counting the microscope optics must completely resolve the grooved lines in block 3 although they may appear somewhat faint, and the grooved lines in blocks 6 and 7 must be invisible when observing them in the center of the graticule area. Blocks 4 and 5 must be at least partially visible but may vary slightly in visibility between microscopes. A microscope which fails to meet these requirements has resolution either too low or too high for fiber counting.
      (3) If image quality deteriorates, clean the microscope optics. If the problem persists, consult the microscope manufacturer.

11. Document the laboratory's precision for each counter for replicate fiber counts.
    a. Maintain as part of the laboratory quality assurance program a set of reference slides to be used on a daily basis. These slides should consist of filter preparations including a range of loadings and background dust levels from a variety of sources including both field and PAT samples. The Quality Assurance Officer should maintain custody of the reference slides and should supply each counter with a minimum of one reference slide per workday. Change the labels on the reference slides periodically so that the counter does not become familiar with the samples.
    b. From blind repeat counts on reference slides, estimate the laboratory intra- and intercounter s_r (see step 21). Obtain separate values of relative standard deviation for each sample matrix analyzed in each of the following ranges: 5 to 20 fibers in 100 graticule fields, 21 to 50 fibers in 100 graticule fields, 51 to 100 fibers in 100 graticule fields, and 100 fibers in less than 100 graticule fields. Maintain control charts for each of these data files.
       NOTE 1: Since fiber counting is the measurement of randomly placed fibers which may be described by a Poisson distribution, a square root transformation of the fiber count data will result in approximately normally distributed data.
       NOTE 2: Certain sample matrices (e.g., asbestos cement) have been shown to give poor precision [6]
12. Prepare and count field blanks along with the field samples. Report counts on each field blank.
    NOTE 1: The identity of blank filters should be unknown to the counter until all counts have been completed.
    NOTE 2: If a field blank yields greater than 7 fibers per 100 graticule fields, report possible contamination of the samples.
13. Perform blind recounts by the same counter on 10% of filters counted (slides relabeled by a person other than the counter). Use the following test to determine whether a pair of counts by the same counter on the same filter should be rejected because of possible bias: Discard the sample if the difference between the two counts exceeds $2.77\ (X)s_r$, where X = average of the two fiber counts and s_r = intracounter relative standard deviation from step 11.
    NOTE: If a pair of counts is rejected by this test, recount the remaining samples in the set and test the new counts against the first counts. Discard all rejected paired counts. It is not necessary to use this statistic on blank counts.
14. Enroll each new counter in a training course which compares performance of counters on a variety of samples using this procedure.
    NOTE: All laboratories engaged in asbestos counting should participate in a proficiency testing program such as the AIHA-NIOSH Proficiency Analytical Testing (PAT) Program and routinely exchange field samples with other laboratories to compare performance of counters.

MEASUREMENT:

15. Center the slide on the stage of the calibrated microscope under the objective lens. Focus the microscope on the plane of the filter.
16. Adjust the microscope (Step 10) [7].
    NOTE: Calibration with the HSE/NPL test slide determines the minimum detectable fiber diameter (ca. 0.25 µm).
17. Select one of the following sets of counting rules:
    NOTE: The two sets of rules have produced approximately equivalent mean counts on a variety of asbestos sample types [6]. OSHA regulations require the use of the A rules [1]. In either case, the rules must be strictly followed to obtain valid results. No hybridizing of the two sets of rules is permitted.

a. A Rules (same as P&CAM 239 rules [2,4,8]; see APPENDIX B).
   1. Count only fibers longer than 5 µm. Measure length of curved fibers along the curve.
   2. Count only fibers with a length-to-width ratio equal to or greater than 3:1.
   3. For fibers which cross the boundary of the graticule field:
      a. Count any fiber longer than 5 µm which lies entirely within the graticule area.
      b. Count as 1/2 fiber any fiber with only one end lying within the graticule area, provided that the fiber meets the criteria of rules a.1. and a.2.
      c. Do not count any fiber which crosses the graticule boundary more than once.
      d. Reject and do not count all other fibers.
   4. Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of a fiber.
   5. Count enough graticule fields to yield 100 fibers. Count a minimum of 20 fields. Stop at 100 graticule fields regardless of count.

b. B Rules (see APPENDIX B)
   1. Count only ends of fibers. Each fiber must be longer than 5 µm and less than 3 µm diameter.
   2. Count only ends of fibers with a length-to-width ratio equal to or greater than 5:1.
   3. Count each fiber end which falls within the graticule area as one end, provided that the fiber meets rules b.1 and b.2. Add split ends to the count as appropriate if the split fiber segment also meets the criteria of rules b.1 and b.2.
   4. Count visibly free ends which meet rules b.1 and b.2 when the fiber appears to be attached to another particle, regardless of the size of the other particle. Count the end of a fiber obscured by another particle if the particle covering the fiber end is less than 3 µm in diameter.
   5. Count free ends of fibers emanating from large clumps and bundles up to a maximum of 10 ends (5 fibers), provided that each segment meets rules b.1 and b.2.
   6. Count enough graticule fields to yield 200 ends. Count a minimum of 20 graticule fields. Stop at 100 graticule fields, regardless of count.
   7. Divide total end count by 2 to yield fiber count.

18. Start counting from the tip of the filter and progress along a radial line to the outer edge. Shift up or down on the filter, and continue in the reverse direction. Select graticule fields randomly by looking away from the eyepiece briefly while advancing the mechanical stage. Ensure that, as a minimum, each analysis covers one radial line from the filter center to the outer edge of the filter. When an agglomerate covers ca. 1/6 or more of the graticule field, reject the graticule field and select another. Do not report rejected graticule fields in the total number counted.

NOTE 1: When counting a graticule field, continuously scan a range of focal planes by moving the fine focus knob to detect very fine fibers which have become embedded in the filter. The small-diameter fibers will be very faint but are an important contribution to the total count. A minimum counting time of 15 seconds per field is appropriate for accurate counting.

NOTE 2: This method does not allow for differentiation of fibers based on morphology. Although some experienced counters are capable of selectively counting only fibers which appear to be asbestiform, there is presently no accepted method for ensuring uniformity of judgment between laboratories. It is, therefore, incumbent upon all laboratories using this method to report total fiber counts. If serious contamination from non-asbestos fibers occurs in samples, other techniques such as transmission electron microscopy must be used to identify the asbestos fiber fraction present in the sample (see NIOSH Method 7402). In some cases (i.e., for fibers with diameters > 1 µm), polarized light microscopy (e.g., NIOSH Method 7403) may be used to identify and eliminate interfering non-crystalline fibers.

CALCULATIONS AND REPORTING OF RESULTS:

19. Calculate and report fiber density on the filter, E (fibers/mm²), by dividing the total fiber count per graticule field, F/n_f, minus the mean field blank count per graticule field, B/n_b, by the graticule field area, A_f (0.00785 mm² for a properly calibrated Walton-Beckett graticule):

$$E = \frac{(\frac{F}{n_f} - \frac{B}{n_b})}{A_f}, \text{ fibers/mm}^2.$$

NOTE: Fiber counts above 1300 fibers/mm² and fiber counts from samples with > 50% of filter area covered with particulate should be reported as "uncountable" or "probably biased."

20. Calculate and report the concentration, C (fibers/mL), of fibers in the air volume sampled, V (L), using the effective collection area of the filter, A_c (385 mm² for a 25-mm filter):

$$C = \frac{(E)(A_c)}{V \cdot 10^3}.$$

NOTE: Periodically check and adjust the value of A_c, if necessary.

21. Report intralaboratory and interlaboratory relative standard deviations (from Step 11) with each set of results.

NOTE: Precision depends on the total number of fibers counted [4,11]. Relative standard deviation (also called coefficient of variation) is documented in references [4,11,12,13] for fiber counts up to 100 fibers in 100 graticule fields. Comparability of interlaboratory results is discussed below. As a first approximation, use 213% above and 49% below the count as the upper and lower confidence limits for fiber counts greater than 20 (Fig. 1).

EVALUATION OF METHOD:

A. This method is a revision of P&CAM 239 [2,4,8]. A summary of the revisions is as follows:
   1. Sampling:
      The change from a 37-mm to a 25-mm filter improves sensitivity for similar air volumes. The change in flow rates allows for 2-m³ full-shift samples to be taken, providing that the filter is not overloaded with non-fibrous particulates. The collection efficiency of the sampler is not a function of flow rate in the range 0.5 to 16 L/min [9].
   2. Sample Preparation Technique:
      The acetone vapor-triacetin preparation technique is a faster, more permanent mounting technique than the dimethyl phthalate/diethyl oxalate method of P&CAM 239 [2,4,5,8,14]. The aluminum "hot block" technique minimizes the amount of acetone needed to prepare each sample.
   3. Measurement:
      a. The Walton-Beckett graticule standardizes the area observed [14,15].
      b. The HSE/NPL test slide standardizes microscope optics for sensitivity to fiber diameter [7,14].

c. An international collaborative study involved 16 laboratories using prepared slides from the asbestos cement, milling, mining, textile, and friction material industries [6]. The modified CRS (NIOSH B) Rules were found to yield equivalent counts but were more precise than the AIA (NIOSH A)* Rules. The relative standard deviations (s_r) varied with sample type and laboratory. The ranges were:

	s_r		
	Intralaboratory	Interlaboratory	Overall
AIA (NIOSH A Rules)*	0.12 to 0.40	0.27 to 0.85	0.46
Modified CRS (NIOSH B Rules)	0.11 to 0.29	0.20 to 0.35	0.25

*Under AIA rules, only fibers having a diameter less than 3 µm are counted and fibers attached to particles larger than 3 µm are not counted. NIOSH A Rules are otherwise similar to the AIA rules.

d. The B Rules have also been favorably received by analysts as less ambiguous and simpler to use; these rules also showed the least bias relative to AIA rules in the collaborative study. An independent NIOSH laboratory study using amosite fibers reported a relative standard deviation, including within- and between-sample variability, of 0.16 for the B Rules [16]. Another NIOSH study was conducted using field samples of asbestos [19]. This study indicated intralaboratory s_r in the range 0.17 to 0.25 and an interlaboratory s_r of 0.45. This agrees well with other recent studies [6,11,13].

e. Because of past inaccuracies associated with low fiber counts, the minimum recommended loading has been increased to 100 fibers/mm² filter area (80 fibers total count). This level should yield intracounter s_r in the range of 0.13 to 0.17 [4,8,16,19].

B. Interlaboratory Comparability:

At this time, there is no independent method for assessing the overall accuracy of this method. One measure of reliability is to estimate how well the count for a single sample agrees with the mean count from a large number of laboratories. The following discussion indicates how this estimation can be carried out based on measurements of the interlaboratory variability, as well as showing how the results of this method relate to the theoretically attainable counting precision and to measured intra- and interlaboratory s_r.

Theoretically, the process of counting randomly (Poisson) distributed fibers on a filter surface will give an s_r that depends on the number, N, of fibers counted:

$$s_r = 1/(N)^{1/2} \qquad (1)$$

Thus s_r is 0.1 for 100 fibers and 0.32 for 10 fibers counted. The actual s_r found in a number of studies is greater than these theoretical numbers [6,11,12,13].

An additional component of variability comes primarily from subjective laboratory-to-laboratory differences. In a study of ten counters in a continuing sample exchange program, Ogden [11] found this subjective component of intralaboratory s_r to be approximately 0.2 and estimated the overall s_r by the term:

$$\frac{(N + (0.2 \cdot N)^2)^{1/2}}{N} \qquad (2)$$

Ogden found that the 90% confidence interval of the individual intralaboratory counts in relation to the means were +2 s_r and - 1.5 s_r. In this program, one sample out of ten was a quality control sample. For laboratories not engaged in an intensive quality assurance program, the subjective component of variability can be higher.

In a study of field sample results in 46 laboratories, the Asbestos Information Association [13] also found that the variability had both a constant component and one that depended on the fiber count. These results gave a subjective interlaboratory component of s_r (on the same basis as Ogden's) for field samples of ca. 0.45. A similar value was obtained for 12 laboratories analyzing a set of 24 field samples [19]. This value falls slightly above the range of s_r (0.25 to 0.42 for 1984-85) found for 80 reference laboratories in the NIOSH Proficiency Analytical Testing (PAT) program for laboratory-generated samples [12].

A number of factors influence s_r for a given laboratory, such as that laboratory's actual counting performance and the type of samples being analyzed. In the absence of other information, such as from an interlaboratory quality assurance program using field samples, the value for the subjective component of variability is chosen as 0.45. Note that, though based on at least two studies, this is a somewhat arbitrary choice. It is hoped that by the use of this number in the absence of other information, laboratories will carry out the recommended interlaboratory quality assurance programs to improve their performance and thus reduce the s_r.

The above relative standard deviations apply when the population mean has been determined. It is more useful, however, for laboratories to estimate the 90% confidence interval on the mean count from a single sample fiber count (Figure 1). These curves assume similar shapes of the count distribution for interlaboratory and intralaboratory results [11].

For example, if a sample yields a count of 24 fibers, Figure 1 indicates that the mean interlaboratory count will fall within the range of 227% above and 52% below that value 90% of the time. We can apply these percentages directly to the air concentrations as well. If, for instance, this sample (24 fibers counted) represented a 500-L volume, then the measured concentration is 0.02 fibers/mL (assuming 100 fields counted, 25-mm filter, 0.00785 mm^2 counting field area). If this same sample were counted by a group of laboratories, there is a 90% probability that the mean would fall between 0.01 and 0.08 fiber/mL. These limits should be reported in any comparison of results between laboratories.

Note that the s_r of 0.45 used to derive Figure 1 is used as an estimate for a random group of laboratories. If several laboratories belonging to a quality assurance group can show that their interlaboratory s_r is smaller, then it is more correct to use that smaller s_r. However, the estimated s_r of 0.45 is to be used in the absence of such information. Note also that it has been found that s_r can be higher for certain types of samples, such as asbestos cement.

Quite often the estimated airborne concentration from an asbestos analysis is used to compare to a regulatory standard. For instance, if one is trying to show compliance with an 0.5 fiber/mL standard using a single sample on which 100 fibers have been counted, then Figure 1 indicates that the 0.5 fiber/mL standard must be 213% higher than the measured air concentration. This indicates that if one measures a fiber concentration of 0.16 fiber/mL (100 fibers counted), then the mean fiber count by a group of laboratories (of which the compliance laboratory might be one) has a 95% chance of being less than 0.5 fibers/mL; i.e., 0.16 + 2.13 x 0.16 = 0.5.

It can be seen from Figure 1 that the Poisson component of the variability is not very important unless the number of fibers counted is small. Therefore, a further approximation is to simply use +213% and -49% as the upper and lower confidence values of the mean for a 100-fiber count.

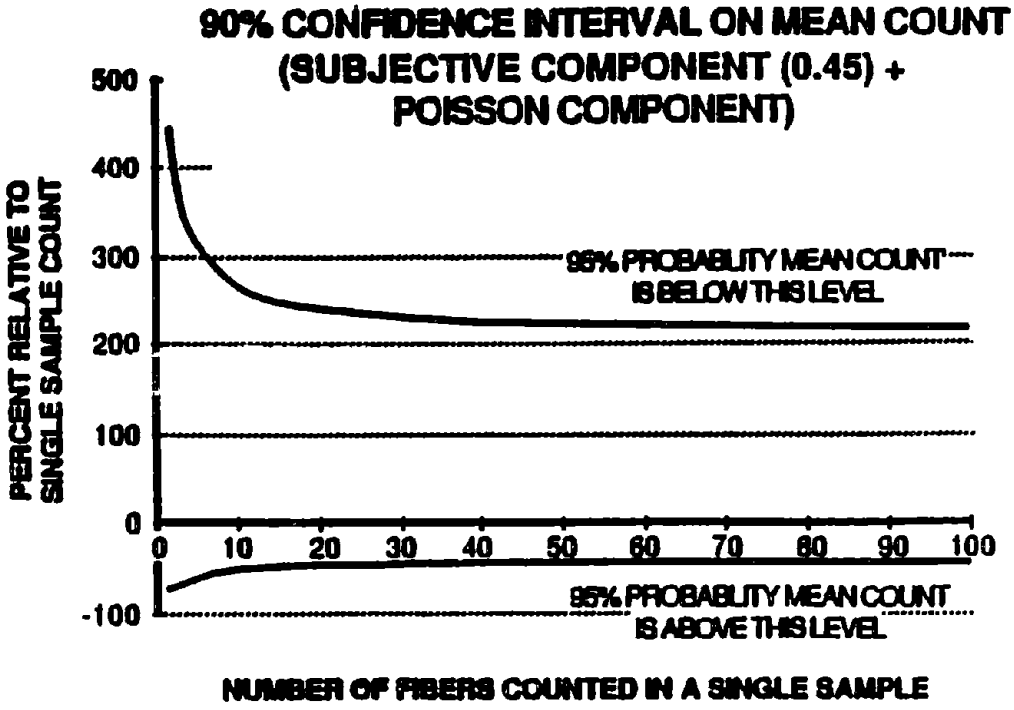

Figure 1. Interlaboratory Precision of Fiber Counts

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METHOD REVISED BY: James W. Carter, David G. Taylor, Ph.D., CIH, and Paul A. Baron, Ph.D., NIOSH/DPSE; based on the revised Method P&CAM 239 [2,4,8].

APPENDIX A: CALIBRATION OF THE WALTON-BECKETT GRATICULE:

Before ordering the Walton-Beckett graticule, the following calibration must be done to obtain a counting area (D) 100 µm in diameter at the image plane. The diameter, d_c (mm), of the circular counting area and the disc diameter must be specified when ordering the graticule.

1. Insert any available graticule into the eyepiece and focus so that the graticule lines are sharp and clear.
2. Set the appropriate interpupillary distance and, if applicable, reset the binocular head adjustment so that the magnification remains constant.

3. Install the 40 to 45X phase objective.
4. Place a stage micrometer on the microscope object stage and focus the microscope on the graduated lines.
5. Measure the magnified grid length of the graticule, L_o (µm), using the stage micrometer.
6. Remove the graticule from the microscope and measure its actual grid length, L_a (mm). This can best be accomplished by using a stage fitted with verniers.
7. Calculate the circle diameter, d_c (mm), for the Walton-Beckett graticule:

$$d_c = \frac{L_a}{L_o} \times D.$$

Example: If L_o = 112 µm, L_a = 4.5 mm and D = 100 µm, then d_c = 4.02 mm.

8. Check the field diameter, D (acceptable range 100 µm ± 2 µm) with a stage micrometer upon receipt of the graticule from the manufacturer. Determine field area (acceptable range 0.00785 mm² ± 0.00032 mm²).

APPENDIX B: COMPARISON OF COUNTING RULES:

Figure 2 shows a Walton-Beckett graticule as seen through the microscope. Although the graticule incorporates the 3:1 aspect ratio, both the "A" and "B" rules will be discussed as they apply to the labeled fibers in the figure.

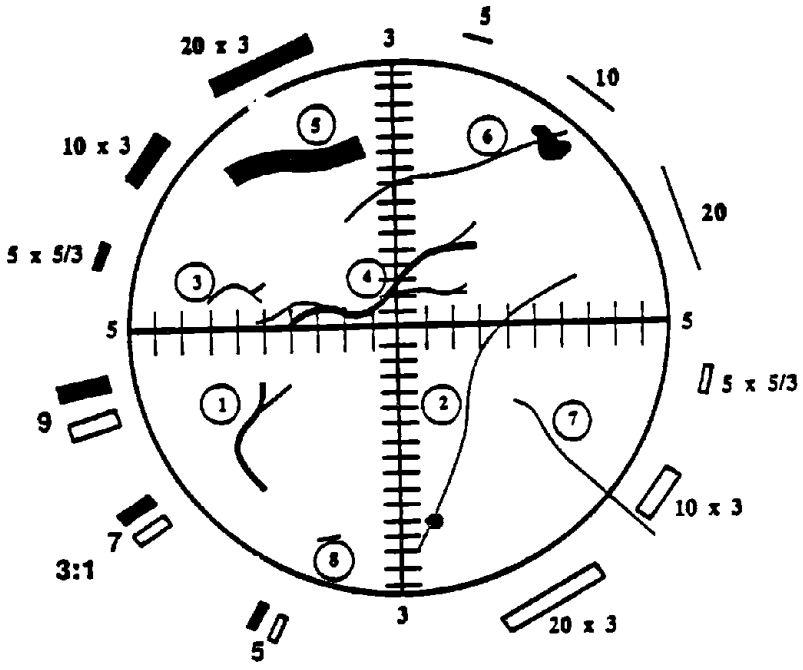

Figure 2. Walton-Beckett graticule with fibers.

FIBER COUNT

Fiber	A Rules	B Rules	DISCUSSION
1	1 fiber	3 ends	(A)"A" rules do not allow for split ends; therefore, count one fiber. (B) Under 'B' rules, first detemine whether the fiber meets dimensional criteria, (i.e., >5 µm, >5:1 aspect ratio, <3 µm diameter). Next determine and count which two ends are the main trunk of the fiber. Finally, count all split ends >5 µm as one end. Fiber #1 is counted as 3 ends.
2	1 fiber	2 ends	(A) Single fiber with small particle attached. The particle is treated as if it does not exist by the "A" rules. (B) The particle is <3 µm diameter and therefore ignored under "B" rules.
3	1 fiber	2 ends	(A) As with Fiber 1, count one fiber under "A" rules because it meets the >3:1 aspect ratio, >5 µm criteria. (B) The split end is <5 µm long so it is not counted under "B" rules.
4	1 fiber	5 ends	(A) Fiber ends all attached to a central large fiber or bundle; therefore, count one fiber under "A" rules. (B) Count two ends as belonging to the main fiber. Three of the remaining four split ends are >5 µm, giving a total of 5 ends.
5	1 fiber	Do not count	(A) No diameter limit under "A" rules; therefore count this thick fiber because it meets the >3:1, >5 µm counting criteria. (B) The fiber is >3 µm diameter; therefore not counted under "B" rules.
6	1 fiber	1 end	(A) Ignore non-fibrous particulate matter under the "A" rules; count this as a whole fiber. (B) The short end of the fiber is <5 µm long and obscured by a particle >3 µm in diameter; therefore, not counted under "B" rules.
7	1/2 fiber	1 end	(A) Fibers which meet rules a.1. and a.2. and cross the graticule boundary are counted as 1/2 fiber under "A" rules unless the fiber crosses the graticule boundary more than once, in which case the fiber is not counted no matter how many ends lie within the graticule area. (B) Fiber ends lying inside the graticule boundary are counted as one end provided that the entire fiber meets rules b.1. and b.2. and each end is >5 µm. The portion of the fiber lying outside the graticule boundary must be considered in order to make this determination. Under "B" rules, it does not matter how often the fiber crosses the graticule boundary.
8	Do not count	Do not count	The fiber is <5 µm long.

Asbestos Analysis - NIOSH Method 7400

Paul A. Baron, Ph.D.

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Introduction: A number of questions regarding the interpretation of fiber counts made with the phase contrast microscopy method (PCM) specified in NIOSH Method 7400[1] have arisen. The information provided here is intended to assist industrial hygienists, analysts and others in interpretation of fiber count results. Note that Revision 3 of NIOSH Method 7400 is included in the second supplement of the NIOSH Manual of Analytical Methods.[1]

The various areas that will be covered below include:

- Discussion of A and B counting rules;
- Requirements for sample loading;
- Differentiation of asbestos vs. non-asbestos fibers;
- Evaluation of samples that are difficult to count;

A vs. B Counting Rules: The two sets of counting rules (designated A and B in NIOSH Method 7400) came about as a result of research conducted by the Institute of Occupational Medicine (Edinburgh, Scotland) under the auspices of the Commission of the European Communities.[2,3] A conclusion of this study was that inter-laboratory variability made a considerably larger contribution to the overall coefficient of variation (CV_t) than any of the other parameters investigated, including the intra-counter and intra-laboratory CV's, and differences between count methods. This study also found that the Central Reference Scheme (CRS) rules developed by the U.K. Health and Safety Executive gave similar mean concentrations, but significantly better inter-laboratory precision than the rules developed by the Asbestos International Association (AIA).[4] The AIA rules were similar to the NIOSH Physical and Chemical Analysis Method (P&CAM) 239 counting rules.[5] As a result of this study, the International Standards Organization (ISO) proposed a method that included both sets of counting rules.

A similar approach was used in the development of NIOSH Method 7400. The NIOSH P&CAM 239 rules were adopted unchanged as the A counting rules while the CRS rules were adopted as the B rules. A notable difference between the two sets of rules was the fact that the B rules used a minimum 5:1 aspect ratio for counting. Although there are analytical reasons for implementing such a ratio as part of the B counting rules, the problems with changing from 3:1 to a 5:1 aspect ratio has led OSHA to require the A rules.[6] NIOSH has recommended the 3:1 aspect ratio in it's policy statements in support of the OSHA standard.[6] On the international scene, a number of countries have adopted the 3:1 aspect ratio rule in their methods, including the European community, Canada and Australia.

Therefore, to eliminate concerns that the two methods are not equivalent when comparing results with the OSHA PEL, it is recommended here that the A rules be used. Also, note that the OSHA regulations also place an upper limit of 2.5 liters/min on the sampling flow rate. Apart from the 3:1 aspect ratio requirement, this is the only other major difference between NIOSH Method 7400 and the OSHA mandatory requirements for measuring occupational exposure to airborne asbestos dust.

Filter Loading Effects: Two parameters in fiber counting that seem to be frequently overlooked are the recommended fiber loading and background dust loading ranges.

The recommended fiber loading range is 100 - 1300 fibers/mm^2. This is the analytical working range over which the count rate remains linear. Above and below this range, significant biases can occur.[7] The bias at lower concentrations is frequently a positive one and may be due to the microscopist's enhanced ability to detect fibers under light particulate loading. At first glance, a positive bias, or counting too many fibers, may seem to be a contradiction. However, the linear working range region of 100 - 1300 fibers/mm2 (or 1 - 5 fibers/field in NIOSH P&CAM 239) has been recommended for many years and is the basis for many legal and health related

measurements. On this basis, one can say that "overcounting" at low filter loadings gives a positive bias. At fiber concentrations above the recommended range, fibers can be obscured by each other as well as background particulate.

The recommended overall dust loading (including fibers and background dust) is less than approximately 50% coverage of the filter surface. Higher dust concentrations result in a negative bias because fibers are obscured by the particulates. The 50% loading level is not a hard and fast rule because obscuration of fibers depends on the size of background particles.[8,9] Small particles will obscure the fibers less than large particles. These biases can be surprisingly high. For instance, Peck, et al., found that with a 60% background coverage of the filter with a silica particulate, the fiber count dropped to 13% of its original value.[9]

It is therefore recommended that samples that do not meet the loadings specified in NIOSH Method 7400 be reported as "not countable." The analyst should report any observed loading levels outside the recommended ranges and indicate:

Underloaded/fibers (may have positive bias);
Overloaded/particulate (may have negative bias);
Overloaded/particulate and fibers (may have negative bias).

If a determination of concentration is needed (e.g:, when a lower limit on fiber concentration is desired), then the results should be reported with one of the notations given above that nonoptimal loading occurred and the results are likely to be biased.

Differentiation of Asbestos vs. Non-Asbestos Fibers: NIOSH Method 7400 is designed to count fibers using PCM and is most often used to count asbestos fibers. Thus, the analyst has a tendency to reject particles not meeting his/her conception of an asbestos fiber. Since NIOSH Method 7400 does not permit differentiation of fiber types, it is improper to reject fibers for reasons other than not meeting the length and aspect ratio requirements. It has been found that even expert microscopists can not make accurate

qualitative determinations of asbestos fibers based on shape or morphology alone.[10] In order to compare fiber concentrations from one analyst to another, it is necessary that all particles meeting the counting criteria of aspect ratio and length be counted as fibers under NIOSH Method 7400. If qualitative analysis is needed, then NIOSH Method 7402[11] (using transmission electron microscopy (TEM)) is recommended. NIOSH Method 7402 provides a means of determining the fraction of asbestos fibers collected on the sample.

There are situations when there is a need to know the fiber type when time or money are not available for TEM analysis. There are other optical microscopy techniques for eliminating some fibers that are not asbestos. Eliminating fibers that are not asbestos from the count made by optical microscopy techniques is termed differential counting. If these techniques are used, then the report of results must clearly indicate which microscopy techniques were applied to obtain the differential count. The most common techniques include the use of crossed polars to identify and eliminate non-crystalline particles. PCM permits the counting of fibers as thin as approximately 0.25 μm, while crossed polars and other qualitative polarized light techniques do not work on fibers thinner than 1 - 2 μm. Note that for many asbestos samples, most of the fiber diameters will be too small to apply the crossed polars technique or other polarized light techniques.

Another differential count technique that can be applied to counts obtained with the A rules is the use of an upper diameter limit of 3 μm. This limit is applied in the B rules and draws its justification from the health effects of asbestos exposure.[12] Asbestos diseases are primarily due to respirable fibers and 3 μm is the upper limit for respirable fibers. Application of this limit will often remove counts of fibrous glass, mineral wool, plant fibers and synthetic organic fibers.

Reports of analysis by NIOSH Method 7400 require counting all particles meeting the aspect ratio and size criteria. The use of

other shape criteria in assessing fiber type is strongly discouraged. This does not preclude the analyst from using differential counting or offering opinions on fiber types present in the sample, but these opinions should not be reflected in the NIOSH Method 7400 fiber count reported. Counts modified by differential counting should be clearly reported as such.

Other optical microscopy techniques (e.g., dispersion staining, extinction angle measurement) can be used, but their quantitative comparability from analyst to analyst is questionable. These techniques require more time and expense for analysis. The technique used must be reported along with the results to indicate that the numbers may not be comparable to other analyses of the same or similar samples. For a more accurate qualitative analysis of fiber concentrations, NIOSH Method 7402 is recommended.

Evaluation of Problem Samples: Counting fibers by NIOSH Method 7400 requires the analyst to make decisions whether or not particles fall within the size and shape limits. If a large fraction of the particles in a sample are near one or more of these limits, then the precision of the results will be poorer. For instance, in samples where large numbers of cleavage fragments occur, many of these fragments will have aspect ratios close to 3:1. Asbestos cement tends to contain many fibers near the 5 µm length limit. Such samples will have increased variability, due to the analyst's difficulty in assessing the criteria on a fiber by fiber basis. This difficulty will also tend to increase analytical costs because of the extra time involved.

Mining environments also often produce another sample type that causes problems with overloading with background dust and large fractions of short, low aspect ratio particles. Asbestos abatement samples produced by aggressive sampling often produce similar samples. At this time, there is no solution to the problem of measuring very low fiber concentrations in high background dust situations. Until better toxicology is available to tell us what specific properties of asbestos fibers are the most important for

health effects, it is unlikely that improvements on the present methods will be available. Although more expensive and time consuming, TEM analysis (NIOSH Method 7402) will often provide a better assessment of fiber exposure for these types of samples, both because qualitative analysis can be performed and because the higher resolution allow a more accurate size and shape of the fibers to be determined. However, because of the complex shapes of asbestos fibers, the precision of TEM counts is not likely to be significantly improved over optical counts.

Conclusions: Several aspects of fiber counting have been addressed. The results are as follows:

1. Use the A counting rules when the results are to be compared with the OSHA PEL.

2. Fiber counts from samples outside the 100 - 1300 fibers/mm^2 range and with more than about 50% of the filter area covered with particulate should be reported as "uncountable" or at least as "probably biased."

3. NIOSH Method 7400 does not allow differentiation of fiber type (asbestos vs. non-asbestos). If optical techniques for differential counting are performed, the results must be reported indicating these differences from NIOSH Method 7400.

4. Optical techniques for qualitative analysis do not work for thin (<1-2 μm diameter) fibers and may vary from analyst to analyst.

5. NIOSH Method 7402 is the recommended procedure for providing data on the fraction of PCM visible asbestos fibers.

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Electrostatic Effects during Asbestos Sampling

Paul A. Baron and Gregory J. Deye
National Institute for Occupational Safety and Health
Division of Physical Sciences and Engineering
Cincinnati OH 45226

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Abstract

Electrostatic Effects during Asbestos Sampling

Paul A. Baron and Gregory J. Deye

One of the factors proposed in recent years as a potential source of error in airborne asbestos fiber measurement is electrostatic charging and interaction between the fibers and sampling cassette. A series of experiments were conducted to measure a) the quantity and location of charge on conductive and non-conductive cassettes, b) the amount of charge developed on fibers generated from a fluidized bed as a function of humidity, c) the modification of particle and fiber deposits on cassettes and filters due to charge effects and d) the effect of various schemes for minimizing charge effects. The results of these experiments have been compared to theoretical calculations of charged particle trajectories under typical sampling conditions.

Most of these experiments and calculations were carried out under the condition of iso-axial, iso-kinetic sampling. Such a condition is generally expected to produce minimum electrostatic effect on the particle trajectories. Some experiments under aniso-kinetic or relatively turbulent air sampling conditions indicate significantly higher losses.

These studies have resulted in a number of recommendations for asbestos sampling. These include: a) that conductive samplers be used to reduce the level of fiber losses during sampling as well as to improve the uniformity of deposit on the filter, b) that an electrical ground on the sampler be used to further reduce potential problems due to electrostatic effects when possible, c) that losses due to electrostatic effects can be quite high under low humidity conditions (<15% RH), d) that concern over electrostatic effects under conditions of moderate to high relative humidity (>30% RH) is probably not warranted, and e) that washing out the interior of the sampling cassette to estimate electrostatic losses is inaccurate.

Introduction

Electrostatic effects are occasionally invoked to explain various anomalies observed in aerosol measurements, especially in the case of asbestos fibers.[1] We have conducted some experiments and calculations to evaluate the effect of electrostatic charge on the sampling and measurement of asbestos fibers. First order electrostatic effects occur when a charge exists both on the particles being sampled and on the sampler. At least three other effects can occur, but will not be addressed below. These include the attraction of a charged particle to a grounded conductive surface and charging of approaching particles with the corona from a highly charged sampler. The former occurs only when the particle is very close to the surface, such as in the small passageways of the lung and the latter occurs with very high voltages on the sampler (probably greater than 15,000 volts) and will result in repulsion of nearby particles.[2] A third effect is the polarization of fibers by the electric field of the sampler. This will cause additional fiber deposition on the inlet increasing with fiber length.

In order to understand the problems that electrostatic charge can cause, one needs to look at the various factors that can enhance or decrease charge on the aerosol and on the sampler. Much of the discussion below applies to compact particles as well, but electrostatic effects are more important for asbestos fiber measurements. Measurements indicate that fibers tend to have higher electrical mobility than compact particles, that is, the fibers tend to move more quickly in an electric field.[2]

In addition, a sampling device is intended to give a representative measurement of the concentration of particles in the air. If, as a particle approaches the sampler, an electrostatic force significantly affects the trajectory of the particle, the sampling device may no longer be accurate. Current asbestos analytical methods dictate that a small but representative area of the filter be viewed, so it is required that the aerosol particles not only enter the sampler in a manner representative of the environment, but that they also deposit quantitatively and uniformly across the filter surface.

Slide 2 depicts the history of a particle from generation to collection. During generation, a fiber is separated from a surface and the process of separation can cause a charge to be left on the fiber. Once in the air, we must consider how particles acquire or lose charge. Finally, during sampling, we need to consider how the charges on both the particle and the sampler affect the trajectories of the particles as they approach and are finally deposited in the sampler and filter.

Experiments, Calculations and Discussion

The magnitude and sign of the charge on the aerosol is a complex function of a number of factors, including the chemical structure of the fiber and of the generation source surface, the amount of water absorbed on the fiber and source surface, the polarizability of the particle, and the speed of separation of the particle from the source surface. Although there have been indications in the literature that low humidity levels can produce high charge levels, there are few reports of the magnitude of the humidity effect.

We have investigated the charge level of asbestos aerosols as a function of humidity for a fluidized bed aerosol generator and this is shown in Slide 3. One can see that the charge level drops approximately an order of magnitude when changing from less than 1% RH to 30% RH. At equilibrium, the water adsorbed on the surfaces is a function of the relative humidity. Thus, the dryness of the generation surfaces can have an important effect on the charge level of the aerosol. Low RH levels can occur in cold climates where indoor air is heated and in desert areas. The water can also be removed from surfaces by heating, so that heated materials may also produce highly charged aerosols.

Particle shape can also affect the amount of charge carried away during generation. Johnston and co-workers at the IOM in Edinburgh have shown that fibers carry a charge proportional to their length and tend to have a higher mobility than compact particles.[2] Measurements of industrial aerosols indicated that fibers were more highly charged than more compact dust particles and that the fibers carried a charge proportional to fiber length.

Once the particle is in the air, the principle means of changing the charge level on the particle is usually due to attraction of oppositely charged ions present in the air. Ions of both polarities are generally present in the atmosphere at concentrations of 10^3 ions/cc because of naturally occurring ionizing radiation.[3] These ions are sufficient to reduce the particle charge 80% in about 10 minutes. Thus, only freshly generated aerosols are likely to carry significant charge levels.

An airborne charged particle moves in an electrical field according to its electrical mobility, that is, the combined effect of the electrostatic force and the air drag resisting particle motion.

$$\text{Electrical Mobility} = neC/3\pi\eta D$$

where n is the number of charges, e is the electronic charge, C is the Cunningham slip correction factor, η is the viscosity of air and D the particle diameter. The motion of a charged fiber in an electric field can be simulated with spherical particles of known charge. Such particles are much easier to generate and measure (as well as simulate theoretically) and many of the following experiments were carried out with these types of particles.

As the aerosol particle approaches the sampler, it can be affected by a charge on the surface of the sampler. As we know, like charges repel and opposite charges attract. In addition, the force of attraction or repulsion is inversely proportional to the square of the distance between the two objects. We have primarily investigated the simplest case of a sampling cassette suspended in space, well separated from other objects, since this is easiest to deal with experimentally and theoretically. From these studies, we can make some extrapolations to actual sampling situations.

Asbestos sampling has been conducted in the past using plastic non-conductive cassettes and is occasionally still done this way.[4] More recently, conductive cassettes have been used, primarily to reduce the sampling error caused by charge.[5] We shall show some of the differences between these types of samplers. Non-conductive samplers can readily acquire and retain randomly distributed charges on their surfaces.

These charges can be of varying magnitude and polarity, producing a complex field that a charged particle must travel through during sampling. The surface charges are produced by triboelectric effect during a rubbing contact with other objects or by coming in contact with other charged objects. These charges can persist on the surface for long periods of time.[6] The degree and the persistence of these charges will tend to be greater under dry conditions. Johnston and co-workers also measured sampling efficiencies under workplace conditions and found only small sampling losses.[2] However, these measurements were made at relative humidities above 30%.

Slide 4 shows some hypothetical particles being pulled through a non-conductive sampler inlet (cowl) to the filter surface. A neutral particle will of course not be affected by the charges on the surface and will be accurately sampled. If some areas of positive charge exist on the surface of the sampler, a negatively charged particle can be attracted to that positive charge. On the other hand, a positively charged particle may be repelled from the inlet surface and land on the filter in an area not representative of the distribution of particles in the air. If the particle carries a larger positive charge, it may be completely repelled from the sampler and not be collected. Thus, the types of effects that one can expect to see on a filter sampling a charged aerosol are losses of particles (producing a negatively biased measurement), especially near the edge of the filter, and an increased variability in the deposit on the filter. This increased variability and bias can especially affect asbestos analysis, since fiber counts are made with the assumption that fibers are uniformly deposited on the filter.

Slide 5 shows a picture of uniformly (all particles carry an identical charge level) charged,[7] dyed particles that have been sampled with two different plastic cassettes. These cassettes were randomly selected from a bag of cassettes. The particles carried a moderately high level of charge (mobility = 0.3 cm^2/statV-sec). The particle deposit is clearly irregular, with virtually complete loss near the filter edges. Note that most naturally generated aerosols carry a distribution of charges and that the shape of the deposit would not be as clearly defined as it is here. This fact, combined with the imprecision of the asbestos fiber count technique, would normally make such an effect difficult to observe.

The reason for the losses near the filter edge and the variability of the deposit can be seen in slide 6. This shows particle trajectories calculated assuming iso-kinetic sampling and charges with random magnitudes randomly distributed on the cowl surface. There are losses near the edge of the filter due to particles attracted to the cowl surface as well as particles pushed away from the cowl walls toward the center of the filter. Because the charges on the cowl surface are randomly distributed, the shape of the filter deposit is irregular.

The conductive sampler situation is somewhat different. Conductive samplers can also become charged by the same mechanisms that produce charge on the non-conductive cassettes. Such charging will also be more likely under low humidity conditions. However, that charge is distributed over the entire cassette surface, tending to reduce the resultant field. In addition, the field produced by this charge will be symmetric. Therefore, the sample deposit of charged particles on the filter will tend to be more radially symmetric.

Slide 7 shows the potential field surrounding an asbestos sampler. This was calculated on a microcomputer spreadsheet using iteration to solve the Laplacian equations.[8] Most of the contour lines are spaced 10 volts apart. The closeness of the contour lines are proportional to the electric field and, hence, to the force that might be applied to an approaching particle. If a positively charged sampler is approached by a positively charged particle under iso-kinetic conditions, Slide 8 shows the trajectories that such a particle would follow. Initially, the particle is repulsed from the sampler as a whole and the trajectories diverge. Near the inlet, the particle feels the effect of the field due to the inlet edge and is focussed toward the center of the inlet. Thus, at sufficiently high charge levels on the particle and sampler, there are losses (negative biases) during sampling.

On the other hand, particles of opposite polarity, as shown in Slide 9 are sampled with nearly 100% efficiency under iso-kinetic conditions. At some distance, the particles are focussed toward the center of the inlet. Near and inside the inlet, the trajectories again diverge and assume a radial distance nearly iso-axial with their starting trajectory. These calculations have been confirmed with experimental measurements as shown in Slide 10. It is clear that there is a drop off in sampling efficiency of similar polarity particles, while there is virtually 100% efficiency and even deposition of opposite polarity particles.

The two filter deposits shown in Slide 10 demonstrate the result of sampling under iso-kinetic conditions with a conductive cassette at 2000 V potential. These filters were also produced with uniformly charged dyed particles as indicated in Slide 5. The opposite polarity particles were uniformly deposited while similar polarity particles were lost near the filter edge.

There are two additional important points to make at this time. First, the particle trajectories are strongly dependent on the amount of time the particles spend in the sampler electric field. Thus, high sampling flow rates will reduce the effects of electrostatic charge. This can be seen in Slide 11. Filter collection efficiency was measured experimentally at one and four L/min for a range of cassette potentials and compared with theoretical calculations.

Second, iso-kinetic conditions do not usually occur during sampling for asbestos. Therefore, particles will approach the sampler on trajectories taking them closer to the sampler walls, thus increasing the losses. Note that deposits from particles on trajectories other than iso-kinetic will tend to be on the outside walls and on the leading edge of the cassette. Sampling performed under more turbulent conditions confirmed significantly increased losses. Under the conditions shown in Slide 11 there was approximately two fold increase in sample loss. However, it was difficult to quantitate the degree of turbulence we had in our sampling configuration.

Up till now the data and calculations discussed have been mostly for spherical particles. Slide 12 shows the fiber deposit on a filter as a function of distance from the edge of the filter. These fibers were fairly highly charged (median mobility = 0.5 cm^2/statV-sec) and confirms that the distributional changes and losses on the filter can be significant for asbestos fibers.

Personal asbestos samplers are placed on the lapel of a worker, with the sampler typically facing slightly outward from the body. If the person's body is electrically charged, this will affect the electric field surrounding the sampler. However, electric fields tend to be highest near sharp objects, so that the field near the sampler inlet is still likely to have a shape similar to the one described above. This phenomenon still needs confirmation. Under area sampling conditions, it is a relatively simple matter to electrically ground the sampler. This will eliminate the charge on the sampler and remove the possibility of an electrostatic interaction.

Suggestions have been made that particle deposits inside a conductive cowl are indicative of electrostatic losses during sampling. As the trajectory calculations and measurements indicated above suggest, very little deposit should occur once a particle is inside the cassette. An experiment was carried out to confirm this hypothesis. A charged, conductive, cowled sampler was used to sample charged chrysotile asbestos fibers. The cowl was carefully removed and cut in half lengthwise with a heated nichrome wire. The cowl halves were then placed in an oven at 50°C and pressed nearly flat. This was accomplished with minimal disturbance of the cowl inner surfaces. These surfaces were then observed with an optical microscope at 100-450X magnification. Virtually all the interior deposit observed occurred in the first millimeter of the leading edge of the cowl. A small deposit was also noted at the edge of the internal lip that supports the cassette cover. Heavy deposits were also noted on the outside of the cowl.

Thus, heavy deposits observed inside conductive cowls are not likely to be produced by electrostatic deposition. It is more likely that such deposits are due to other factors such as direct contamination, impaction and settling of large particles, or disturbance of deposit on the filter surface.

Another commonly used remedy for electrostatic charges on non-conductive surfaces has been application of anti-static sprays. A variety of these sprays have been tried. They typically contain a liquid that evaporates quickly, leaving a slightly conductive residue on the surface. This can be quite effective in reducing localized charging. However, some of these materials can be removing by rubbing, while others can be washed off with isopropyl alcohol. With proper application and care, the use of cassettes sprayed with anti-static material can be an effective alternative to conductive cassettes.

Conclusions (Slide 13)

Electrostatic effects can occur in sampling asbestos fibers and other aerosols, especially under dry conditions for freshly generated aerosol. Plastic non-conductive cassettes can carry high and non-uniform charges on their surfaces, resulting in negative biases and increased variability of deposit in the sample. Conductive samplers will tend to give a more uniform and less biased sample. The fiber deposit on the inlet, or cowl, of conductive samplers will occur primarily on the exterior and leading edge of the cowl. Measurement of the deposit in the interior of the cowl will probably not give a good indication of electrostatically deposited fibers. Under area sampling conditions, grounding the sampler will virtually eliminate electrostatic effects on the aerosol.

The measurements and calculations described here give an indication of type and magnitude of effects one might observe when electrostatic charge is a significant factor during sampling. For instance, heavy fiber deposits at the leading edge of the inlet and losses primarily near the filter edge are both indicators of electrostatic charge effects. The occurrence of these effects may be difficult to detect during routine analysis of industrial hygiene asbestos samples because the method is not designed to detect them.

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Slides

1. Title.

2. Charge effects during aerosol particle life.

3. Humidity dependence of fiber charge.

4. Schematic view of charged particle sampling.

5. Visual deposit of particles sampled with non-conductive sampler.

6. Calculated trajectories of particles sampled with a non-conductive cowl with several randomly placed charges on the cowl surface.

7. Iso-potential surfaces surrounding a conductive cassette. Note: The surfaces depicted here are in error to due to an error in boundary conditions. The surfaces should be approximately 50% further from the cassette surface, although the shape, especially near the inlet is approximately the same. The trajectory calculations were carried out with the correct potential field.

8. Calculated trajectories of particles sampled with aconductive cowl with the potential field indicated in Slide 7. The particles have the same polarity as the cowl.

9. Calculated trajectories of particles sampled with a conductive cowl with the potential field indicated in Slide 7. The particles have a polarity opposite to that of the cowl.

10. Visual deposit of particles sampled with non-conductive sampler.

11. Comparison of theoretical and experimental sampling efficiencies for particles of a single size and charge as a function of conductive sampler potential and flow rate.

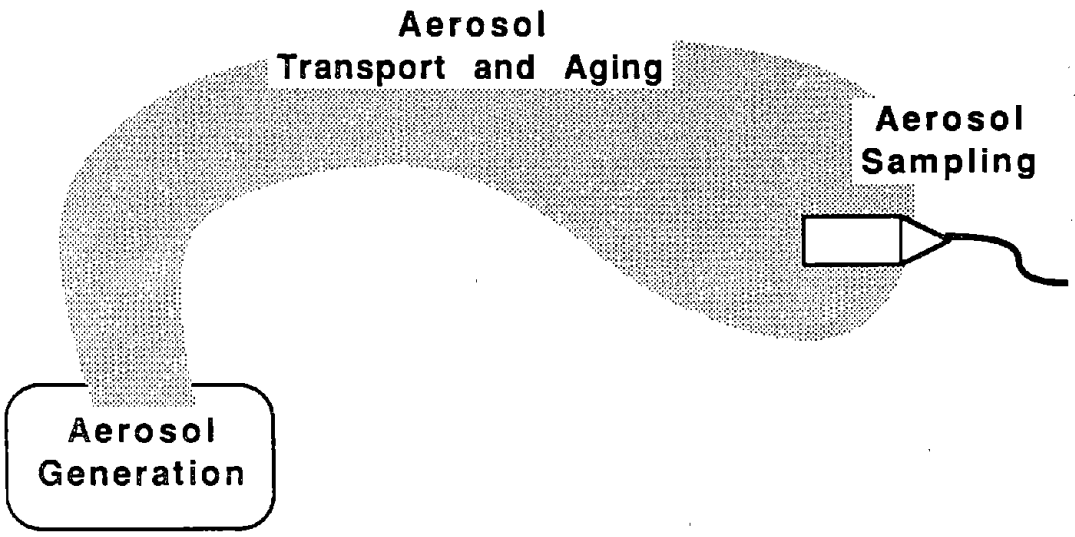
12. Filter deposition efficiency of a conductive samplers as a function of filter radius for unipolar chrysotile fibers.

13. Conclusions.

Electrostatic Effects in Asbestos Fiber Sampling

Paul Baron and Gregory Deye

National Institute for Occupational Safety and Health
Cincinnati OH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Sampling
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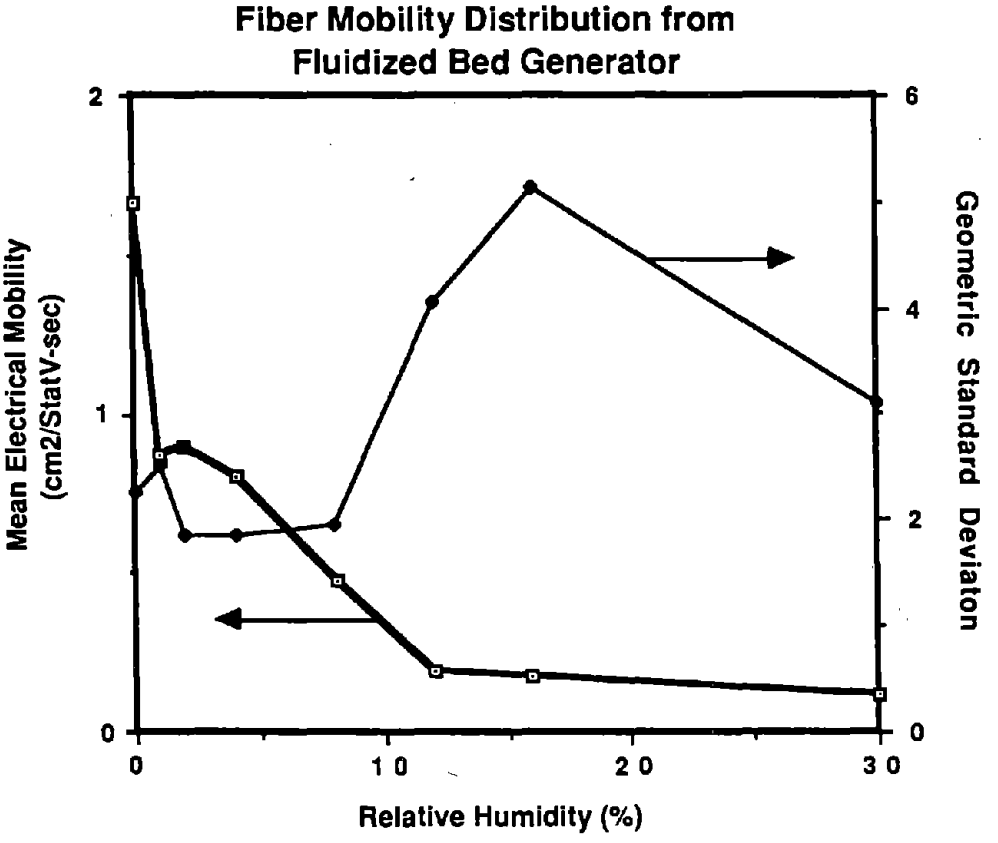
Fiber Mobility Distribution from
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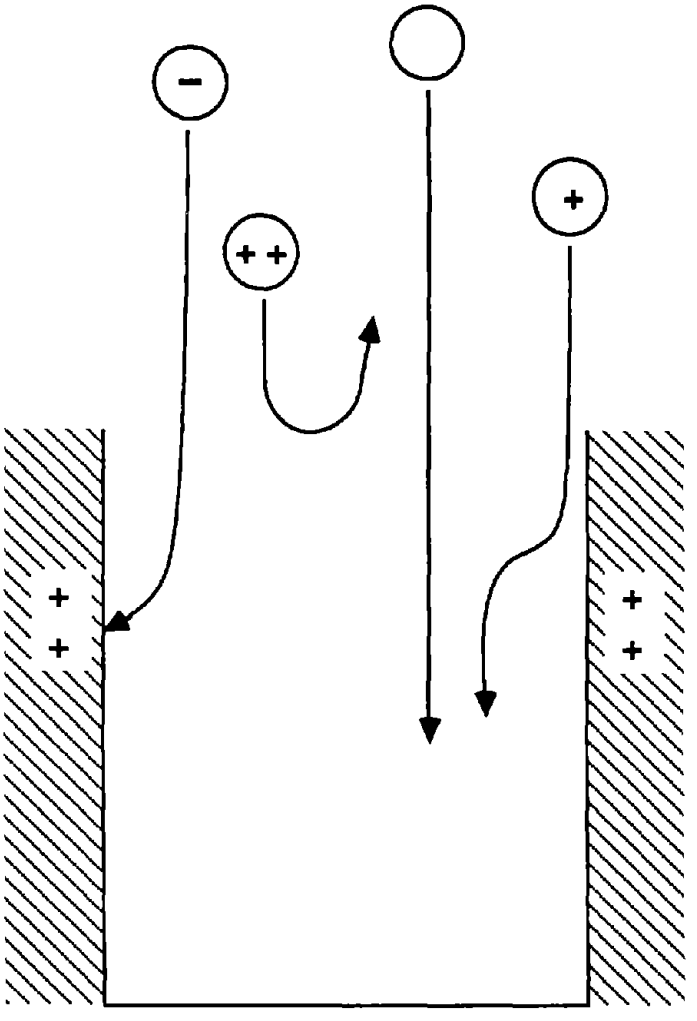
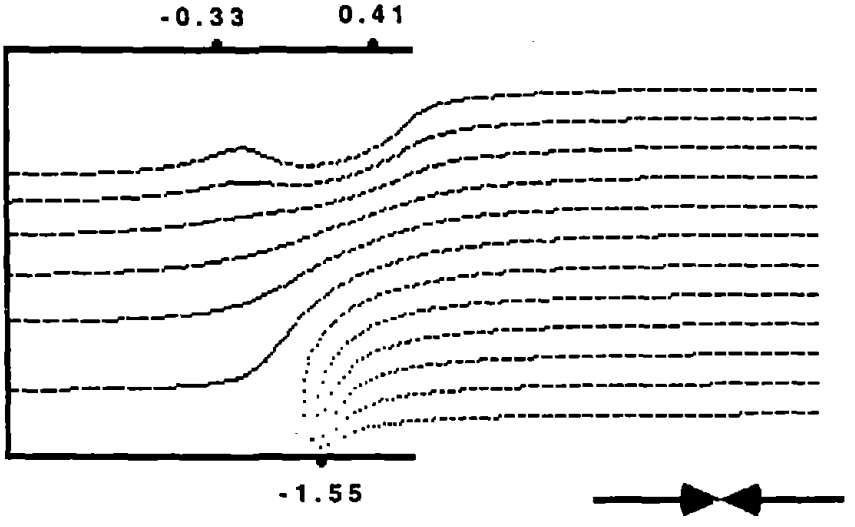


Sampling Charged Particles with a Non-conductive Cassette

Filter Deposits of Particles Sampled with Non-Conductive Cassette

$$\text{Particle Mobility} = -0.3 \frac{\text{cm}^2}{\text{statV-sec}}$$

Particle Trajectories Calculated for a Non-conducting Cassette with Several Randomly Distributed Charges

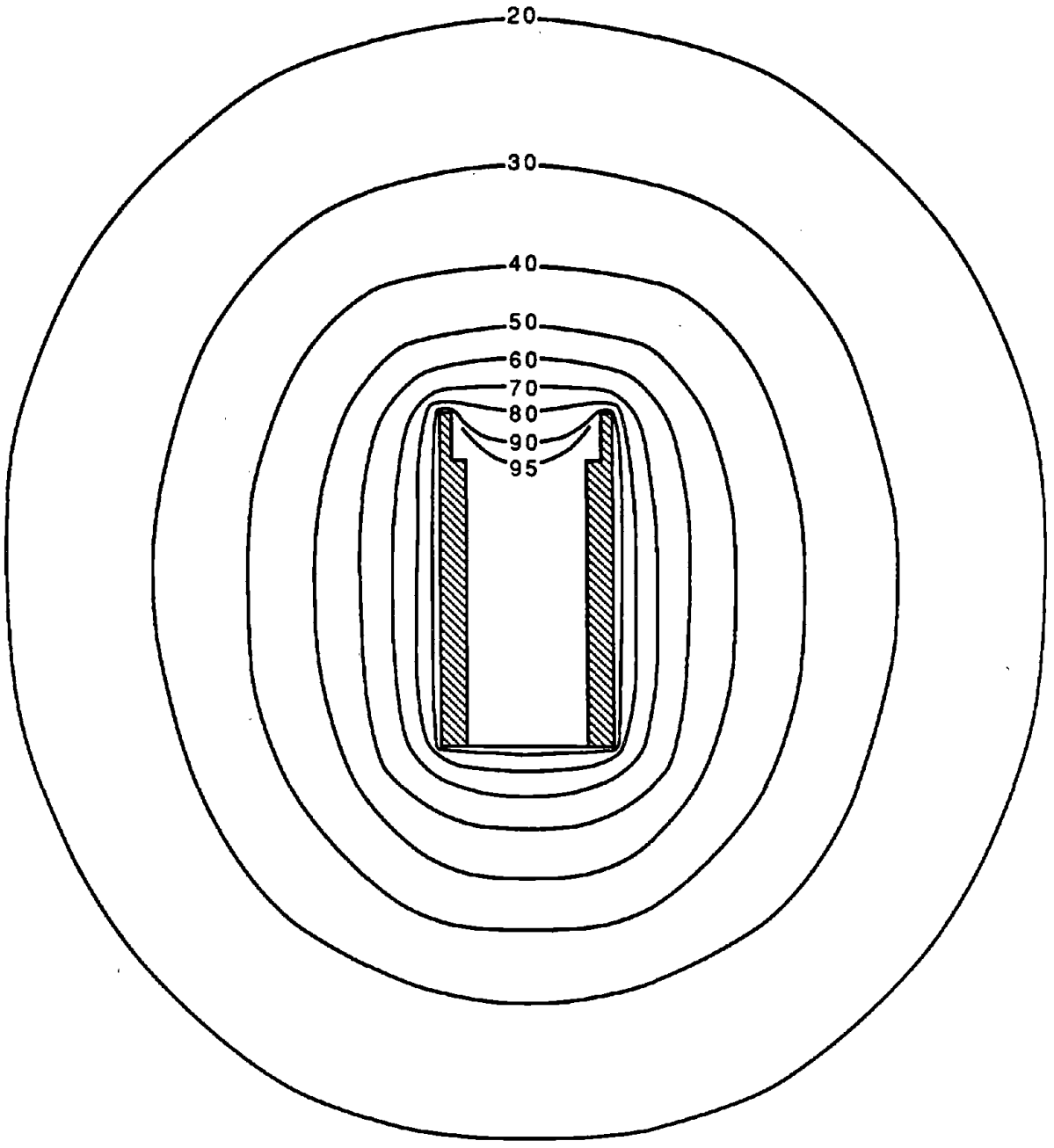


Particle Charge = 1500 unit charges

Particle Diameter = 5.0 μm

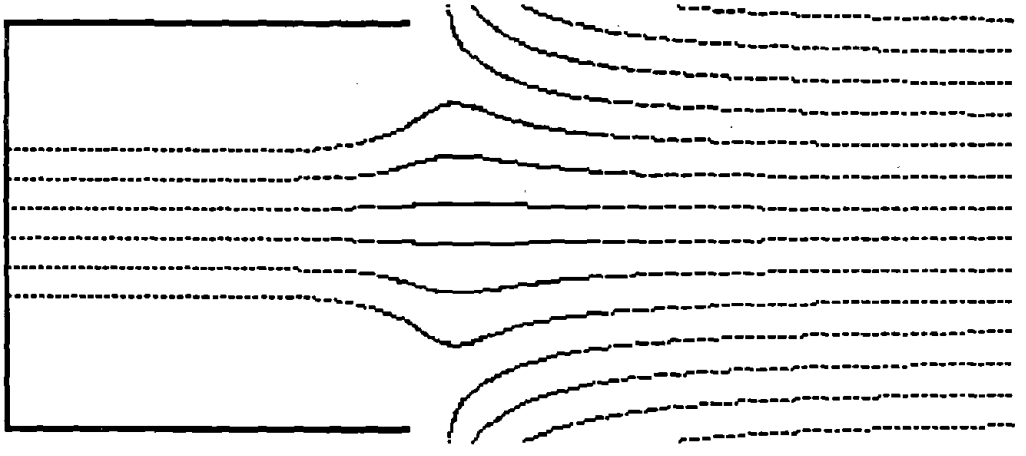
Flow Rate = 1.0 L/min

Wall Charges x 10^{-8} unit charges



Potential Field Lines Around An Isolated Conductive Cassette At 100 V

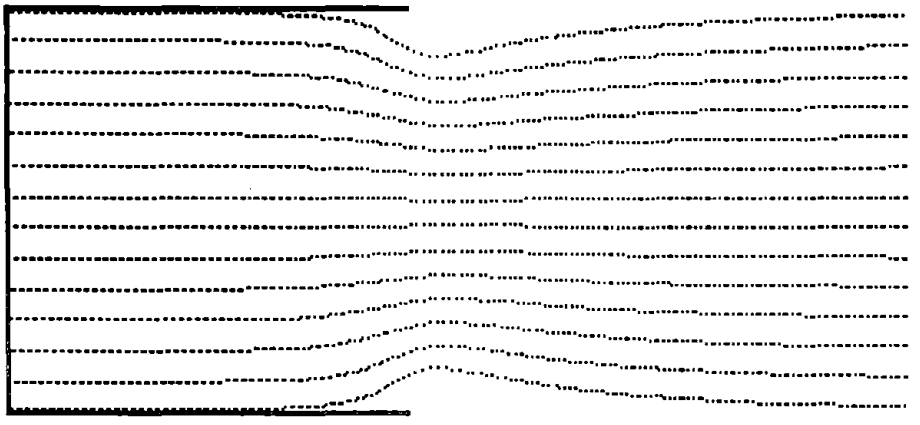
Particle Trajectories Calculated for a Conducting Cassette Same Polarity (particle/cassette)



Particle Charges = 1500
Cassette Voltage = 3000 volts
Particle Diameter = 7.2 μm
Air Velocity = 0.74 L/min

Scale compressed by X2

Particle Trajectories Calculated for a Conducting Cassette Opposite Polarity (particle/cassette)



Particle Charges = 1500

Cassette Voltage = -3000 volts

Particle Diameter = 7.2 μm

Air Velocity = 0.74 L/min

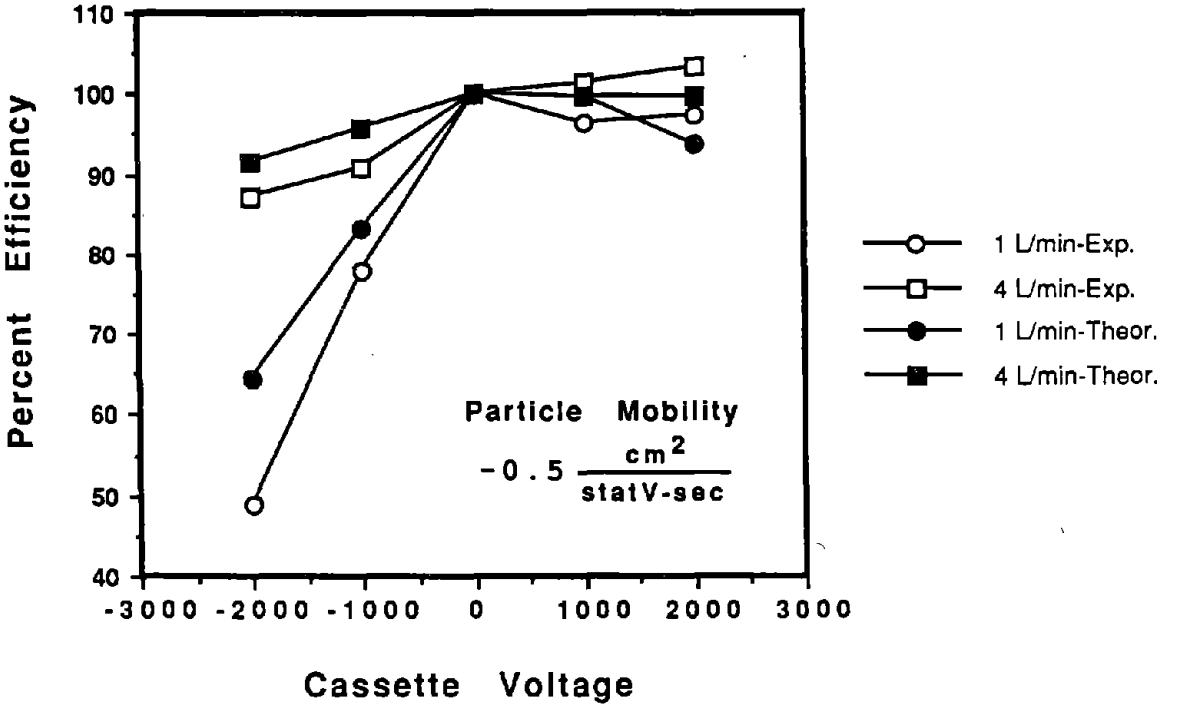
Scale compressed by X2

Cassette Voltage = -2500 volts Cassette Voltage = 2500 volts

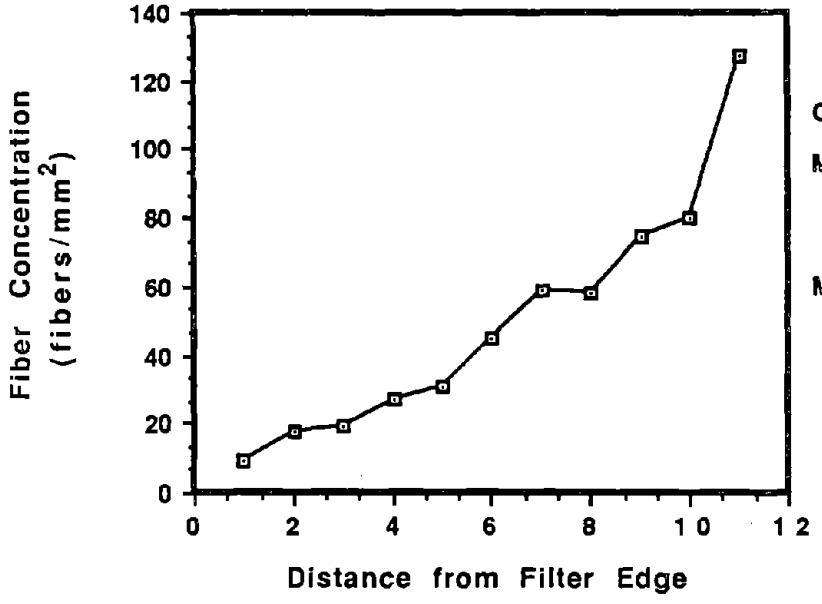
$$\text{Particle Mobility} = -1.2 \frac{\text{cm}^2}{\text{statV-sec}}$$

Filter Deposits of Particles Sampled with Conductive Cassette

Theoretical vs. Experimental
Filter Collection Efficiency for Conductive Sampler



Radial Distribution of Charged Fibers Collected with Conductive Cassette



Cassette Voltage = 2500 volts

Mobility Range = -0.2 to 1.4 $\frac{cm^2}{statV\text{-}sec}$

Median Mobility = 0.5 $\frac{cm^2}{statV\text{-}sec}$

Conclusions

- Fiber charge can increase by an order of magnitude at low humidity levels
- Freshly generated fibers will carry the highest charge levels
- Non-conductive samplers can produce filters with high losses and high variabilty
- Conductive samplers will tend to have lower biases and less variability on filter
- Charge effects will decrease at higher flow rates
- Grounding sampler and nearby surfaces will further reduce electrostatic effects

Pump Calibration Laboratory

Stephen G. Bayer

March 1988

By performing at least three independent measurements on at least four points on a rotameter scale, calibrate a personal-sampling pump.

Given filter specifications and flow rate, test the automatic flow-compensation feature to ensure that a selected flow rate will remain within ± 5%

Determine the degree of reproducibility to be expected when a flow rate is adjusted from the calibration information.

* CALIBRATION *

The determination

within specified limits (precision)

of the true values (accuracy)

of the indications on a rotameter

Disclaimer

This manuscript is intended for use as instructional material only. Mention of equipment or materials, by brand name, does not constitute endorsement by NIOSH. The content of this module is not to be construed as an official report of performance characteristics, except as it relates to classroom purposes when comparing results within the NIOSH direct 582 course.

AIRFLOW CALIBRATION STANDARDS

PRIMARY

A container having a fixed volume that can be determined by direct measurement, in some fashion independent of the gas or air involved in the calibration process, is a primary air-flow calibration standard. The two most common examples are SOAP-FILM BURETTE and WATER-FILLED SPIROMETERS. The spirometer has a typical capacity of six liters but is not practical for field calibration. The burette is portable but has a practical maximum capacity of one or two liters.

INTERMEDIATE

Instruments which demonstrate accuracy and precision similar to a primary standard (but do not meet the definition of a primary) are considered intermediate standards. Intermediate standards are better than secondary standards; but, intermediates must be periodically calibrated by comparison to a primary. Examples are WET-TEST METERS and DRY-GAS METERS. Both have the advantage of dial readouts of air volume meaning that larger volumes can be measured as compared to the lesser volume capacities associated with most primary standards. Thus, higher flow rates can be determined with improved accuracy and precision.

SECONDARY

An instrument which can be used for flow-rate determinations but does not meet either of the above criteria is considered a secondary standard. Most secondaries are flow-indicating or flow-metering devices. Examples include ROTAMETERS and ORIFICES. A set of calibrated orifices could be used to calibrate several points on a rotameter, for example. Also, one rotameter could be used to calibrate another; however, such practices are not good practice- unless no other means is available.

Certain rules of good practice

1) Always measure as large of an air volume as possible.

2) Average more than one reading, preferably three at any one rotameter number.

3) Include readjustment of the ball in each of the three trials.

4) When reading a rotameter:

   A) Make sure gravity is pulling straight down on the ball.
   B) Keep the ball at eye level.
   C) Place the center of the ball across the line.
   D) Mentally average the bounce or jiggle.

5) Field calibration is usually a quick investigation of the rotameter number of interest. Field calibration is typically performed daily (i.e. before and after).

6) Laboratory calibration is a thorough investigation including four points on the rotameter scale and a constant-flow pressure drop test.

7) When performing the constant-flow pressure-drop test, begin with a suction value typical for the rotameter setting and the filtration medium used. Then, increase the suction in approximately 2" H_2O increments WITHOUT readjusting the pump. You may reach a point at which the flow is no longer within 5% of the original value. Note that the rotameter ball may no longer float in its' original position. If not, the rotameter changed calibration!

8) The system being calibrated should include the filter, cassette, typical length of tubing, etc. representative of the actual sampling system. The sampling system should be connected to the calibrator in such a fashion so as to minimize any extra resistance.

Rotameter Calibration Data

FOR TRAINING PURPOSES ONLY

Equipment identity and desc. _____

_____

Calibrator identity and desc. _____

_____

Name: _____ Date: __/__/__

Temp: _____ Baro pr. _____

rotameter number	vol L	suction "H_2O	time trials time #1	time #2	time #3	av time sec	flow rate L/min
#							
#							
#							
#							

$$\text{Flow Rate} = \frac{\text{Meas Vol L}}{\text{Average Sec}} \times \frac{60 \text{ Sec}}{1 \text{ Min}} = \frac{\text{L}}{\text{Min}}$$

$$\text{Actual Flow} = \text{Calibrated Flow} \sqrt{\frac{P_{cal} \times T_{act}}{P_{act} \times T_{cal}}}$$

P = Barometric Pressure mm Hg T = Temperature Kelvin (°C + 273)

Pump #90780

25 mm Nuclepore 1.2 um + 50 mm cowl

ROT #	L/min	"H_2O		
3.5	3.0			
	2.9	15+		
3.2	2.7	13		
	2.7	13		
3.0	2.5		2.45	12
	2.5	14	2.4	12
	2.6	12		
	2.7	11		
2.5	2.2	10		
	2.0	10		
	2.1	9		
2.0	1.7		1.7	8
	1.7	10	1.7	8
	1.7	7		
	1.8	8		
1.5	1.4	6	1.3	6
	1.3	6	1.3	6
	1.3	6		
1.0	0.88			
	0.86	4		
	0.90	4		
0.8	0.65	2	0.70	3
	0.70	3	0.60	3
	0.65	3		

Pump #90780

37 mm Millipore 0.8 um 2 filters

3.0	2.42	14
2.0	1.65	9
1.5	1.27	7
0.8	0.66	3.3

Pump #90780

37 mm Millipore 0.8 um no cowl

ROT #	L/MIN	"H_2O		
3.5	3.2	9		
3.2	2.7	10		
	2.74	10		
3.0	2.6	8	2.6	8
	2.6	7	2.56	8
	2.6	9	2.61	8
	2.6	8.6		
2.5	2.0	5		
	2.2	7		
	2.1	7		
2.0	1.7	4	1.7	5
	1.8	4	1.68	5
	1.7	5	1.7	5
	1.7	5.3		
1.5	1.36	4	1.33	4
	1.33	4	1.30	3
	1.30	4	1.35	3.5
	1.30	4.1		
1.0	0.90	2		
	0.92	2		
0.8	0.64	2	0.66	2
	0.63	2	0.65	2
	0.65	2	0.62	1.5
	0.65	1.8		

Pump #90780

25 mm 0.45 um Millipore w/cowl

Rot#	L/min	"H_2O
3.0	2.2	36
2.5	1.9	30
2.0	1.7	24
1.0	0.8	13

Pump #90780

37 mm 5.0 um PVC

Rot#	L/min	"H_2O
3.0	2.6	4.5
2.0	1.7	2.6
1.5	1.3	2.0
0.8	0.66	0.9

37 mm pad only

3.2	3.0	2
2.4	2.2	1.5
1.6	1.5	1.5
0.8	0.65	1

25 mm pad only

3.0	2.5	4
1.7	2.0	2
1.0	0.88	1
0.8	0.66	<1

Pump #90781 25 mm Nuclepore 1.2 um WITH COWL

ROT #	L/MIN	"H2O	L/min	"H_2O
3.5	3.0	17		
3.4	3.2	17		
3.2	2.8	13	2.9	13
	2.8	14	2.8	12
	2.8	13		
3.0	2.6	13	2.6	13
	2.6	14	2.58	12
	2.5	12	2.57	11
	2.5	12	2.6	12
	2.5	12		
2.5	2.2	10	2.1	10
	2.1	10	2.1	10
	2.1	10		
2.0	1.7	9	1.7	9
	1.8	10	1.73	8
	1.74	8	1.72	8
	1.7	8	1.8	8
	1.7	8		
1.5	1.4	6	1.3	6
	1.4	6	1.3	6
	1.4	6	1.3	6
	1.33	6	1.30	5
	1.37	6	1.4	6
	1.3	6		
1.0	0.91	4		
	0.93	5		
	0.89	5		
0.8	0.70	3	0.69	3
	0.76	3	0.73	3
	0.73	3	0.69	3
	0.68	3	0.70	3
	0.71	2	0.70	2
	0.80	2		

Pump #90781

37 mm Millipore 0.8 um

ROT #	L/MIN	"H_2O
3.2	2.8	11
	2.8	11
3.0	2.6	11
	2.7	9
	2.6	9.5
	2.6	9.5
	2.56	8.7
2.5	2.2	8
2.4	2.2	8
2.0	1.8	7
	1.9	6
	1.75	6
	1.8	6
	1.75	5.5
1.6	1.5	5
1.5	1.4	4
	1.4	4
	1.32	4
1.0	0.93	3
	0.90	2.5
	0.94	3
0.8	0.73	2
	0.72	2
	0.80	2
	0.71	2
	0.73	2

Pump #90781

25 mm 1.2 um with cowl
2 filters in cassette

Rot #	L/min	"H_2O	
3.2	2.6	15+	(beyond meter range)
	2.65	21	
3.0	2.4	20	
	2.44	20	
2.5	2.1	15	
2.0	1.7	14	
	1.69	14	
	1.71	14	
1.5	1.3	10	
	1.3	11	
	1.32	10.5	
	1.31	10	
0.8	0.7	5	
	0.7	6	
	0.71	5.5	
	0.70	5.9	

Pump #S-39 (HETAB)

37 mm MCEF 0.8 um 3 piece

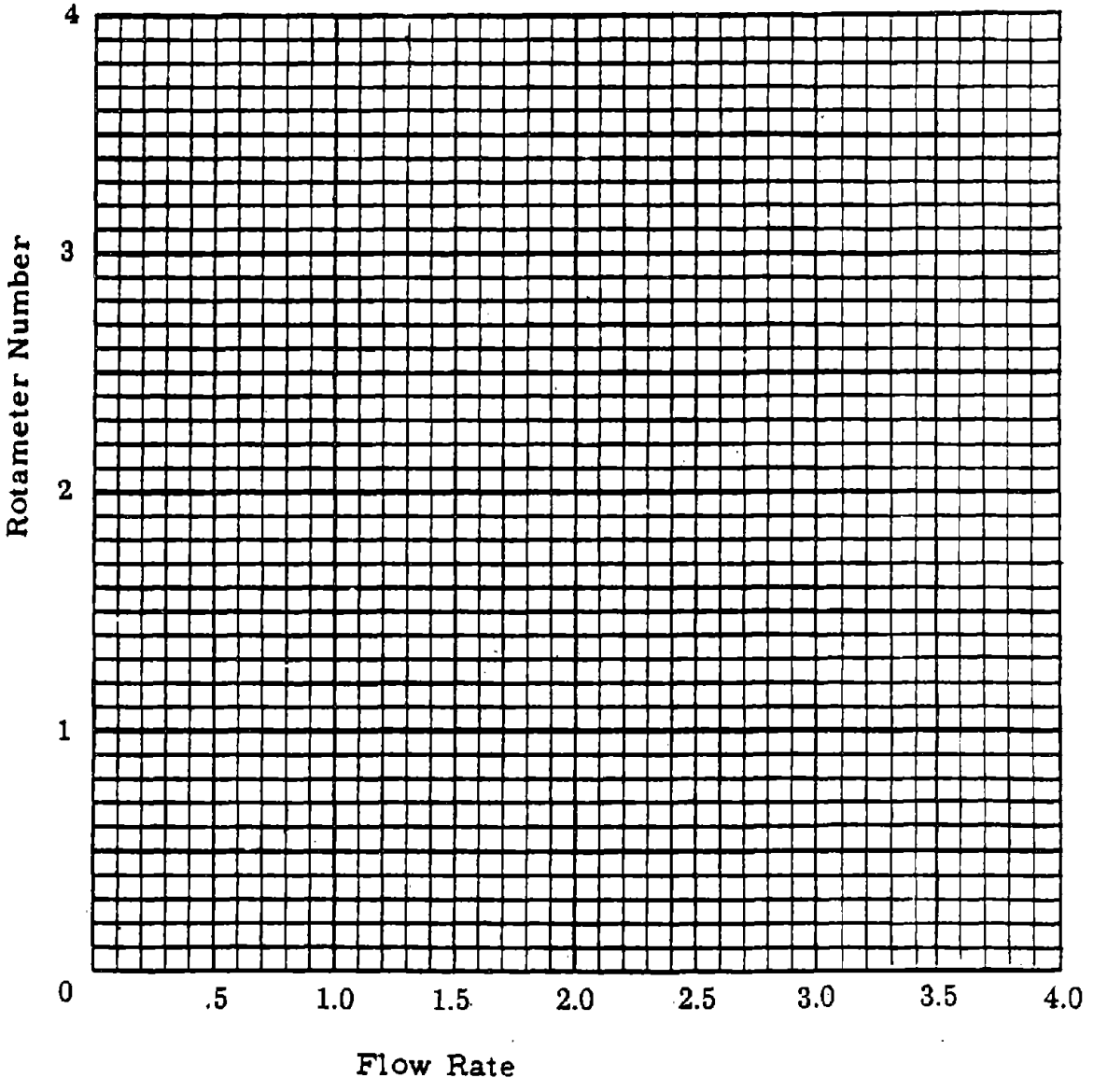
Rot #	L/min
3.0	2.6
	2.6
2.0	1.7
	1.7
1.5	1.3
	1.3
0.8	0.62
	0.60

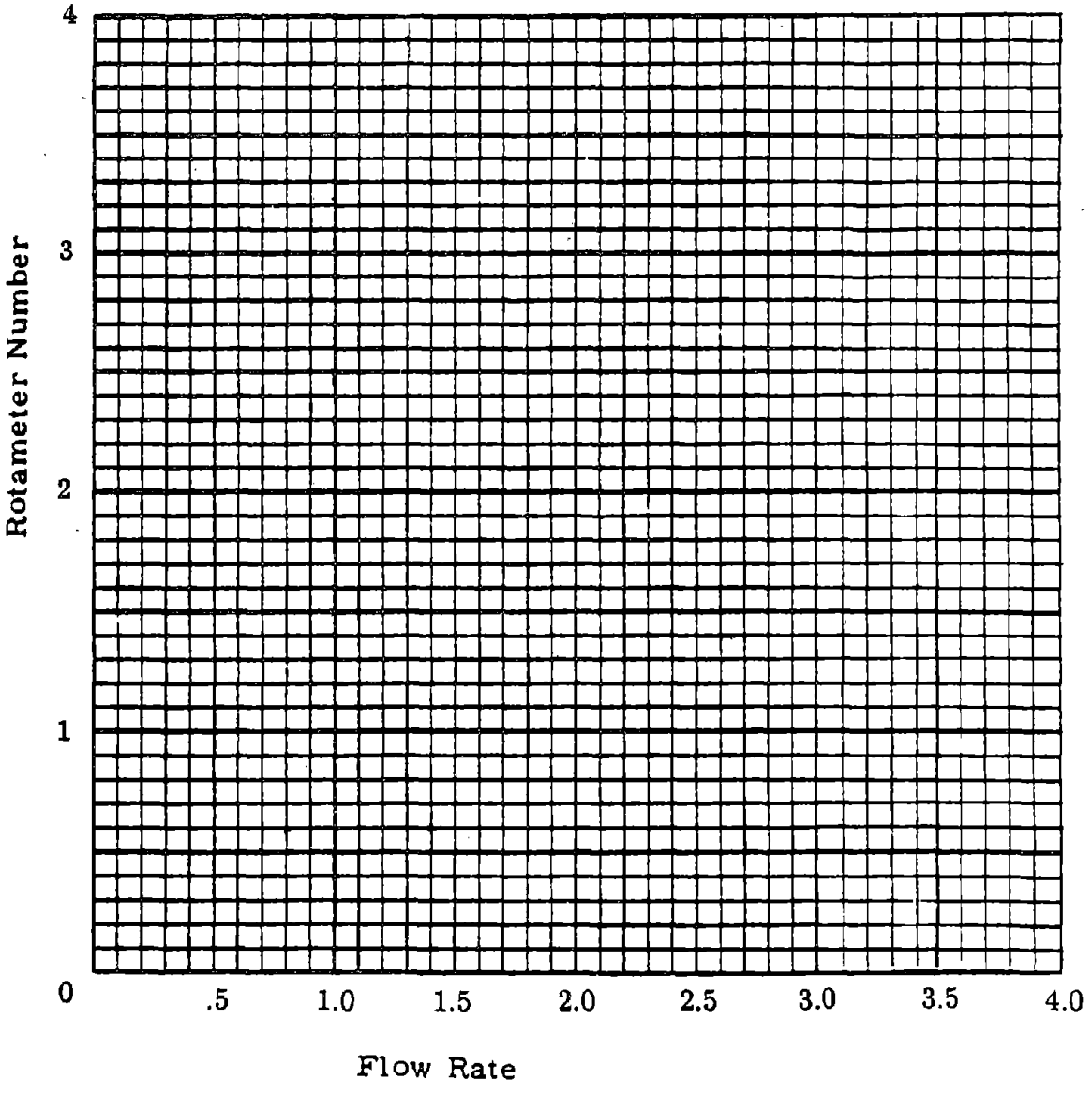
*** FLOW-RATE REPRODUCIBILITY TESTS ***

The student is assigned a "goal" flow rate. The desired rotameter setting is interpreted from the calibration graph prepared in the laboratory. The student adjusts the pump accordingly and a soap film is timed. The actual flow rate is calculated and reported versus the goal. Earlier data has the flow rounded to the nearest 0.1 L/min. Later data is rounded to the nearest 0.01 L/min, more appropriate for comparative purposes.

LITERS/MINUTE

Goal			Actual		
0.7	0.705	0.70			
0.8	0.63	0.82			
1.0	0.93	1.03	1.0	1.09	
1.1	1.1	1.18	1.0		
1.2	1.15	1.21			
1.3	1.1	1.36	1.22		
1.4	1.37	1.40	1.40		
1.5	1.43	1.56			
1.7	1.7	1.72	1.76		
1.8	1.76	1.81			
1.9	2.0	1.88	1.84	1.93	1.43
2.0	2.0	2.0	1.97	1.90	
2.3	2.1	2.38			
2.4	2.3	2.7	2.4	2.52	
2.5	2.53	2.6	2.53		
2.8	2.7	2.73	2.79	2.73	


Rotameter Number
4
3
2
1
0
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Flow Rate


Rotameter Number
4
3
2
1
0
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Flow Rate

Asbestos Sampling Heuristics

(Rules for Good Guessing)

Version #2

APRIL 1988

by Stephen G. Bayer

Disclaimer

This module is part of the instructional materials used in the NIOSH direct-training 582 course. This manual insert contains the author's explanations, opinions and viewpoints and has not undergone NIOSH manuscript clearance procedures. Therefore, the content is not intended for use outside the NIOSH direct course and should not be interpreted as universal NIOSH policy.

Please do not, in any official capacity, quote from nor reference this document. Instead, quote and reference appropriate sections of the information and publications given in the recommended reading section at the end of this module.

* Simplified Concentration Equation *

$$\text{Concentration} = \frac{\text{Average Count} \times \text{Sampling Area}}{\text{Field Area} \times \text{Flow Rate} \times \text{Sample Time} \times \text{Conversion}}$$

Units: NOTE-- references to "fibers" assumes fibers longer than 5 micrometers!
references to "Method" refers to NIOSH Method 7400

Concentration: fib/mL (fibers per milliliter of air)

Average count: fib/fld (fibers per field)

Sampling Area: mm^2 (square millimeters)

Field Area: mm^2/fld (square millimeters per field)

Flow rate: L/min (liters per minute)

Sample time: min (minutes)

Conversion: 1000 mL/L (1000 milliliters per liter)

Using dimensional analysis, the concentration in fibers per milliliter is calculated thus:

$$\frac{\text{fib}}{\text{mL}} = \frac{\frac{\text{fib}}{\text{fld}} \times \frac{mm^2}{1}}{\frac{mm^2}{\text{fld}} \times \frac{\text{L}}{\text{min}} \times \frac{\text{min}}{1} \times 1000\ \frac{\text{mL}}{\text{L}}}$$

Fibers remain on the top and milliliters remain on the bottom; everything else cancels out: the concentration is expressed as fibers/milliliter. The conversion factor may be expressed in cubic centimeters (cc or cm^3), generating a concentration in fibers/cc! Since the OSHA standard is in fibers/mL, this module standardizes on milliliters.

Expected Variable Values

The concentration equation, from the previous page, ignores the blank's average count since the purpose of this module is to select sampling parameters. Typical values for each of the variables in the equation can be generalized:

1) Average Count: 0.785 - 10.2 fib/fld
100 - 1300 fib/mm^2 Count Ranges

2) Filtration Area: 385 mm^2 (25 mm filter- standard)
855 mm^2 (37 mm filter- unique occasions)
Note: Use measured values.

3) Field Area: 0.00754 - 0.00817 mm^2 (Walton-Beckett tolerance)
0.00785 mm^2 (proper)

4) Flow Rate: 0.5 - 16.0 L/min (method 7400)
0.5 - 2.5 L/min (OSHA reference method)

5) Sampling Time: 15 - 480 min (typical OSHA-related monitoring)
15 - unlimited (general ambient monitoring)

6) Concentration: Depends on count and sampling values

The concentration and all other variables in the equation are mathematically related. Just as a certain sample volume and count will determine a given concentration, a given concentration and sample volume should generate a certain count. A given count, for a selected concentration, determines a required sample volume.

The sample volume is determined by multiplying the flow rate, the sample time, and 1000 to obtain the volume (in mL). For sampling purposes, do not multiply by 1000: leave the volume in liters. In practice, one usually selects either the time or the flow rate and then calculates the unknown variable. For a given concentration, there are three areas of interest:

Sampling Considerations

1) What should the count be?

2) What flow rate should be drawn?

3) How long should the sample be taken?

The starting concern should be the count. Controlling the count in the sampling process cannot be understated.

Relating Count Units

The unit fib/mm^2 is related to the count and the field area. It is a term of fiber density, determined by dividing the average count by the field area. Multiplying the fiber density by the field area calculates an average count. On occasion, a total fiber count may be expressed (i.e. 78.5 fibers). When the count is LESS THAN 100 (not equal to or greater than!) one can reasonably assume that one hundred fields have been observed and mentally divide the count by 100 to obtain an average.

Thus, for a standard field area of 0.00785 mm^2/fld, a "count" can be described, for example, in three ways: 5.5 fibers counted, an average count of 0.055 fib/fld, and a fiber-density of 7.0 fibers/mm^2. The equations are:

$$\text{Average Count} = \frac{\text{\# fibers observed}}{\text{\# fields observed}} = \frac{\text{fibers}}{\text{field}}$$

$$\text{Fiber Density} = \frac{\text{average count} \quad \text{fib/fld}}{\text{field area mm}^2\text{/fld}} = \frac{\text{fibers}}{\text{mm}^2}$$

$$\text{Average Count} = \text{Fiber Density} \ \frac{\text{fib}}{\text{mm}^2} \ \text{X Field Area} \ \frac{\text{mm}}{\text{fld}} = \frac{\text{fib}}{\text{fld}}$$

LOD and LOQ

The Limit of Detection (LOD) is prescribed at 7 fib/mm^2. The Limit of Quantitation (LOQ) is 100 fibers/mm^2.

The Limit of Detection (LOD) is a value representing the smallest amount of material that can be confidently distinguished from background levels. The LOD is generally defined as a value three times higher than that expected from a typical blank. In other words, one could normally expect a blank to have around one to one and a half fibers seen in one hundred fields.

However, if a sample's count comes very close to a blank's count, one is not sure whether the count is "true" and due to the sample or whether the count is "false" from a heavier-than-normal blank.

Thus, a blank may actually have one to three fibers/one hundred fields; but (from a statistical perspective), the count may be expected to range up to five fibers/hundred fields. If the count could be expected to go as high as five, one would need to see at least five and a half fibers in one hundred fields to ensure that a fiber had been DETECTED! Therefore, the Method expresses the LOD as an estimated value.

The Limit of Quantitation (LOQ) is an amount of analyte at which a certain acceptable level of precision has been reached. The minimum specified count range of 100 fib/mm^2 is the value to use as an LOQ.

The range over which the variability should be acceptable and linear (i.e. predictable) is 100 - 1300 fib/mm^2. This is termed the "working range".

Below 100 fib/mm^2, the variation increases and becomes less predictable. There is a tendency to overcount, producing what is termed "positive bias." That means that the resultant concentration tends to measure higher than the concentration is.

When counts exceed 1300 fib/mm^2, overcrowding becomes a problem, distinguishing one fiber from several becomes difficult, and particulates tend to obscure fibers. The effect is a general reduction in count which, similar to lighter samples, demonstrates an increase in variation and a negative bias. Negative bias means a measured concentration lower than the true value.

Interfering particulates are a problem. A general rule, described in the Method, is than when 50% of the area of the visible surface of the sample is covered with particles, the filter is too overloaded to accurately count.

For the purposes of sampling heuristics, in the absence of prior experience with the dust cloud, one must assume no particulate interference. In other words, this module illustrates a means of selecting sampling parameters which will assist in producing measurements with improved precision. This module is offered as an approach for a starting point in sampling. There is no substitute for on-the-job experience in gauging effective sampling parameters and no substitute for initial analytical data which helps fine tune future sampling parameters.

When selecting sampling parameters:

Goal

One should select a sample volume that produces 100 - 1300 fibers/mm^2.

The count is important: the number of fibers counted has an effect on the accuracy of the analysis and the precision of the measurement. Accuracy is the ability to obtain a correct measurement and precision is a measure of how well a measurement can be reproduced. An excellent method of measurement will produce an accurate (correct) measurement and the precision will be high (i.e. a very low coefficient of variation or relative standard deviation).

Count

To begin with sampling heuristics, assume the count to be the minimum needed: 100 fibers/mm^2. In terms of the concentration equation, this would be a count of 78.5 fibers seen in 100 fields (averaging 0.785 fibers/field with a field area of 0.00785 mm^2/field).

Next,the concentration:

1) The concentration might be estimated from previous analytical data on the specific sampling circumstance

2) The concentration might be estimated from historical analytical data from similar environments or operations.

3) The concentration might be totally unknown.

In classification #1, confidence in the selected sampling parameter would be very high, classification #2 would produce some uncertainty in the selection of parameters, and classification #3 would generate a low degree of confidence.

Suppose one was expected to determine if an individual, working with asbestos, was in compliance with the OSHA standard.

Which standard? OSHA has a Permissible Exposure Limit of 0.2 fib/mL (PEL) and an Action Limit (AL) of 0.1 fib/mL. In the case of a company determining compliance status, one would expect an attempt to establish that the standard has not been violated. Thus, expect a concentration less than the standard. With an unknown concentration, one would not use the standard (PEL or AL) as a starting point. One would probably select a lower value. Since exceeding the action limit triggers increased monitoring and more, a company would prefer the concentration to be below 0.1 fib/mL and be able to demonstrate compliance status.

Assume that the concentration is less than the action limit: 0.025 fib/mL, for example. So: — Concentration Rule

1) Keep the count above 0.785 fibers per field

2) Assume the concentration to be 0.025 fib/mL

Next, one has to decide on flow rates and sampling times. Statistically, two samples to prove one fact are far superior to one, all else equal. Thus, two four-hour samples would be preferred, if possible. More time may be needed. For the purposes of the first exercise:

3) The required sampling time is unknown — Determine the Time

The lower the concentration, the higher the flow rate. This is a helpful philosophy. The ORM calls for a maximum of 2.5 L/min. Use it! — Maximum Flow

4) Flow rate is 2.5 L/min

The field area is assumed to be 0.00785 mm^2/fld and the filter area is assumed to be 385 mm^2. — Standard Areas

Recalling the equation on the first page:

$$\text{fib/mL} = \frac{\text{average count X filtration area}}{\text{field area X flow rate X time X 1000}}$$

$$0.025 = \frac{0.785 \text{ X } 385}{0.00785 \text{ X } 2.5 \text{ X min X } 1000}$$

Algebraically, the concentration and sample time exchange places:

$$\text{min} = \frac{0.785 \text{ X } 385}{0.00785 \text{ X } 2.5 \text{ X } 0.025 \text{ X } 1000}$$

$$\frac{302}{0.491} = 616 \text{ minutes}$$

Thus, if the sample was taken for 616 minutes at 2.5 L/min, with a standard field and filtration area, one would expect a count of 0.785 fib/fld when the concentration is 0.025 fib/mL. One cannot sample for four hours and remain above the LOQ.

What would the count be if one samples for for only four hours? Use this equation:

Average Count

$$\text{fib/fld} = \frac{\text{conc X field area X flow X time X 1000}}{\text{filter area}}$$

To provide a sample (at one fourth the action limit) having an average count equal to the LOQ, one would need to sample for 616 minutes: a lesser time would result in a lower average count which would, in turn, reduce the precision of the analysis and reduce the confidence placed on the measurement.

The count (for a four-hour sample) would be:

$$\text{fib/fld} = \frac{0.025 \times 0.00785 \times 2.5 \times 240 \times 1000}{385} = 0.31$$

Instead of the preferred average count of 0.785, the average would be 0.31 fibers per field or 31 fibers seen in 100 fields-- less than the LOQ. From the graph (Figure 1, top line) in section B of Method 7400, the concentration resulting from 31 fibers in 100 fields could be 230% higher than the measured value!

0.025 fib/mL X 2.3 = 0.058 fib/mL

Statistics

By using Equation 2, in section B of the Method, the estimated overall S_r (CV) would be:

$$S_r = \frac{(31 + (0.2 \times 31)^2)^{1/2}}{31} = 0.27$$

Everything on the top line is raised to the 1/2 or 0.5 power: this means take the SQUARE ROOT! Statistically, applying the Null hypothesis to one sample taken, the concentration could be:

Establish Compliance

95% UCL (one sided) = measurement + (1.645 X S_r X standard)

0.025 fib/mL + (1.645 X 0.27 X 0.1) = 0.069 fib/mL

The standard used above is the air quality standard for which one is trying to establish compliance. The measurement is the concentration resulting from analysis. The S_r (CV) was determined from Equation 2 in section B. Using parameters selected earlier, both the graph and the equation in section B demonstrate that an actual count of 31 fibers in 100 fields show that the action limit (0.1 fib/mL) has not been violated (from a statistical viewpoint and measured concentration of 0.025 fib/mL).

If sampling for 4.00 hours at 2.5 L/min, limit of detection (LOD) as a concentration could be calculated. Recall that the estimated LOD is 7 fib/mm^2 (calculated by dividing the average count by the field area). One could revise the concentration equation to:

LOD Concentration

$$\text{Conc} = \text{fib/mm}^2 \times \frac{\text{filter area}}{\text{flow} \times \text{time} \times 1000}$$

Notice that average count and field area have both been replaced by a value in fib/mm^2.

$$\text{Conc} = \frac{7 \times 385}{2.5 \times 240 \times 1000} = 0.0045 \text{ fib/mL}$$

One could say that a concentration of 0.0045 fib/mL or higher is measurable. A MAJOR PROBLEM is that someone with little scientific understanding of measurement variability may think that measurements at or near the limit of detection are as valid as higher measurements. This is a misconception.

The following, based on the previous example, is a summary of concentration values resulting from sampling at 2.5 L/min for 240 minutes:

$$\text{Conc} = \frac{7 \times 385}{2.5 \times 240 \times 1000} = 0.0045 \text{ fib/mL}$$ LOD

$$\text{Conc} = \frac{100 \times 385}{2.5 \times 240 \times 1000} = 0.064 \text{ fib/mL}$$ LOQ

$$\text{Conc} = \frac{1300 \times 385}{2.5 \times 240 \times 1000} = 0.83 \text{ fib/mL}$$ Maximum

To stay within the working range of the Method (particulate interference absent),2.5 liters per minute for 240 minutes measures concentrations between 0.064 and 0.83 fibers/milliliter.

This example assumed that a company is taking a sample for the purpose of establishing compliance with the OSHA Action Limit of 0.1 fib/mL. When determining sampling parameters (when trying to establish that one is IN compliance), use a concentration value as far below the standard as one can measure while trying to keep the count well above 7 fib/mm^2 (preferably above 100 fib/mm^2).

A different example is based on establishing that a standard has been violated.

Determining Violations

The OSHA PEL is 0.2 fib/mL based on an 8 hour time-weighted average (8-hr TWA). Two 4 hour samples would be delightful; however, many work a 3.5 hour morning and a 4.5 hour afternoon. The flow rate, in the ORM, is limited to 2.5 L/min. Remember that a compliance-related official would have interest in whether or not the Action Limit has been exceeded.

One consideration might be: "what concentration value would exist at the LOQ if one were to sample for 3.5 hours at 2.5 L/min?"

$$\text{Conc} = \frac{100 \text{ X } 385}{2.5 \text{ X } 210 \text{ X } 1000} = 0.073 \text{ fib/mL}$$

What would be a maximum concentration for the above parameters which would fall within the specified count range?

$$\text{Conc} = \frac{1300 \text{ X } 385}{2.5 \text{ X } 210 \text{ X } 1000} = 0.95 \text{ fib/mL}$$

Thus, any concentration between 0.073 fib/mL and 0.95 fib/mL would be within the working range of the Method. The specified range of count produces a concentration measurement range thirteen times higher than the lowest selected concentration.

Time-weighted Average

The concentration measured, for any single sample, is an integrated average representative of the period of time over which the sample was taken. In the unlikely event that someone works for eight consecutive hours (and was sampled during the full eight hours), the measured concentration from a full-period sample would represent the entire day: time-weighting would not be needed.

Seldom is that the case: typically, many work nine hours which includes a half hour lunch and two fifteen minute breaks. Two samples, one before and after lunch, are usually required. Keeping the sample times exactly the same is impractical. If the sample times were exactly the same, one could directly average the two measurements. Time weighting is simply a mathematical process used when the sample times are <u>different</u>.

Samplers are expected to become fatigued after about six hours using the standard (1.2 um, 25 mm diameter) filter. Industrial hygienists also have to work (at least) nine hour days to routinely collect eight hour's worth of samples. Thus, one could expect most sample periods to be somewhat less than eight hours.

These common real-life difficulties lead to some general philosophies regarding 8 hour time-weighted averaging concepts.

Most people work eight hour days: most toxicological data assumes that the body has sixteen hours to eliminate and/or detoxify the poison. Therefore, the average concentration for a typical (representative) workday is important. One <u>cannot</u> average measurements directly when the sample times are <u>different</u>. The time-weighted average must integrate both the measured concentrations and the times of the individual samples.

The equation for time-weighted averaging is:

Equation

$$\text{Conc}_{TWA} = \frac{C_1T_1 + C_2T_2 + C_3T_3 + \ldots + C_nT_n}{\text{Total Sampling Time}}$$

"C" symbolizes concentration. The numbers (1, 2, 3, etc.) are the first, second, third, etc. consecutive samples taken on one day (to "n" numbers of samples). The samples are taken on the same day on a given individual or specific location within an environment. "T" represents sample time: the units must be the same top and bottom. Total sampling time is the sum of the individual times.

If the total time is eight hours, one has an 8-hour TWA. If not, then one does not. The TWA is representative only of the total period sampled. If the total time is 5.5 hours, one has a 5.5 hour TWA.

The purpose of an 8 hour TWA is to represent the day. Data, by definition, is a collection of information representing fact. Sampling must be representative. Here are several commonly asked questions concerned with measuring TWA's:

Question #1

What if an employee works in an asbestos-suspect area for only four hours and the remainder of the day is elsewhere. Can I divide by eight to get the TWA?

Answer: Yes, if one can demonstrate that the remaining time period is <u>free</u> of asbestos. If in doubt, sample during the worker's other four hours. Having data to back up assumptions pays off. Data provides facts and eliminates assumptions.

Question #2

I hear that OSHA divides by eight regardless of the total time period. Someone told me that industry should do things just like OSHA does. Should I always divide by eight?

Answer: No! A quick experiment will demonstrate that dividing by eight (when the total time is less than eight hours) always reduces the TWA. If OSHA needs to substantiate that a violation and the sampling time is less than eight hours, OSHA will likely divide by eight. (Note: For information about OSHA policy, contact your local OSHA office!) OSHA regulations stipulate eight-hour TWA's. How could OSHA prove a violation without having one? In such a case, dividing by eight gives the company the benefit of doubt, mathematically assuming the the concentration during the unsampled period was ZERO! A company following such a philosophy would always be giving themselves the benefit of doubt.

What then, should I assume if the total sampling time is less than eight hours? Question #3

Answer: In the absence of other information, assume the sampled period to be representative of the whole day.

What is the typical time span considered by most to be the <u>minimum</u> that should be representative of eight hours? Question #4

Answer: About six hours!

Can't professional judgement be used to provide conclusions in the absence of actual data? Question #5

Answer: Certainly. one can use judgement. The problem is: can the judgement be supported? What credentials, experience, and the like does one have in order for the judgement to be accepted as fact? The primary difference between assumptions and judgements is that assumptions are not based on sound facts.

Next comes fashions in which the measurements can be interpreted and/or reported.

Reporting Results

There are several problems associated with reporting data. One set of information is needed for asbestos-related specialists, another is needed for those specializing in other somewhat-related professions (such as architects, engineers, etc.), and often a third set of interpretations is needed for far-removed occupations (such as laborers, building owners, etc.)

Light Counts

When counts are less than the LOQ (100 fib/mm^2), one can expect two phenomena. First, the filter is quite large relative to the area observed. One hundred standard counting fields constitute only 0.2% of the filter. One hundred fields counted equates to 0.8% of the mounted filter segment which, in turn, is representative of the entire filter.

Especially with chrysotile asbestos, one might expect some "clumping" or areas of higher density here and there on the filter. One individual might have a total count of fifteen fibers (having seen no clumps) and another person might count the filter and see twice as many fibers having hit a couple of clumps. Both counters, all things equal, are correct. One could say that they are functioning under the "luck of the draw".

The fewer fibers there are on the filter, the more likely it is that a light count will result. A light count by one person does not guarantee the same light count by another.The lighter the count, the more relative variation there is when the difference in the total count is, for example, five fibers. There is little relative difference between 95 and 100: there is a large relative difference between 5 and 10. The difference is the same: the difference is simply proportionally larger with the smaller counts. Such an example produces a high relative standard deviation on the low count. All things equal, one expects a rise in the variation (a decline in precision) when the count declines.

With light samples (less than the limit of quantitation in other words), one expects three things to happen. First, one expects an increase in the disagreement between two independent counts-- an increase on analytical variability. Second, with a declining count, one expects the variation to increase (precision suffers). Furthermore, it is difficult to predict just how bad the variation will be because the variability becomes variable.

Not Countable

Thus, when the fiber density is less than 100 fib/mm^2, Dr. Paul Baron (see suggested reading) suggests that filters not meeting the loadings specified in the method be reported as "not countable". The following statement should qualify the measured concentration when someone is insistent upon a concentration value:

"Underloaded/fibers (may have positive bias)"

In a conversational manner, one could make a statement similar to the following:

"There were too few fibers present to state the concentration with a good degree of confidence. The actual concentration may be less than than that which is indicated in this measurement."

In addition, one should clearly state that the measurement resulted from a count outside the method's recommended count range.

Interferences
Fibers

One form of interference is simply too many fibers present-- above 1300 fib/mm^2. In this region of fiber density, fibers tend to overlay one another and distinguishing one from another and/or gauging size becomes difficult.

The outcome is a reduction in the actual concentration producing an artificially low measurement. This error or inaccuracy,on the low side, is termed a "negative bias". With the relatively low concentrations present in recent years, fiber overloading without particulate overloading would not be likely. If such was the case, the following qualifier would be appropriate:

"Overloaded/fibers (may have negative bias)"

In plain English:

"This concentration resulted from a filter crowded with fibers. Measuring the exact number was not possible. The true concentration is probably higher than indicated by this analysis."

Interferences
Particluates

The fiber count may be within the specified count range but much (the method states 50%) of the filter surface is covered by particles. In a similar fashion, the count is artificially reduced. Naturally, adding more fibers compounds the issue.

In a similar fashion, Dr. Baron suggests:

"Overloaded/particulate (may have negative bias)

or

"Overloaded/particulate and fibers (may have negative bias)"

Again, the negative bias indicates that the measured concentration could underestimate the true concentration.

Summary

The number of fibers and particulates present have a distinct effect on the accuracy (or correctness) of a given count and the precision (reproducibility or agreement)of multiple evaluations (by the same individual or by another) of the same filter.

"Negative bias" means the measurement is lower than the true concentration. The concentration should be considered "greater than —— fib/mL".

"Positive bias" means the concentration is higher than the true value. The resultant concentration should be reported as "less than —— fib/mL".

Of course, there are many real-life situations where particulate interferences are extreme. A recent example involved sampling for low fiber concentrations in a tunnel beneath toll booths. When sampling a volume appropriate for the fiber-count perspective, the exhaust fumes were totally obscuring the filter surface!

When the air volume is reduced so one can see between the exhaust-fume particles, the result is likely to be a nonexistant fiber count.

Last, but not least, is that reporting the measured concentration requires statistical considerations not covered in this module.

This chapter provides information for determining appropriate flow rates and sample times to help collect good samples. The decisions are based upon the mathematical interrelationships of concentration, count, sampling area, field area, flow rate, and sample time.

Selection of these parameters requires knowledge on how each is affected by the other and how all affect the precision and accuracy of the method. To collect the best sample (for analysis of a given filter), the prime consideration is striving for the best possible count, considering the sample volume and the concentration of interest.

To establish that one is in compliance, in the absence of other information, select a concentration value which is, at the very most, one half of the applicable standard. A very light count will mean that the concentration is in compliance. As the count becomes higher with increasing concentration, the variability will decline and help make decisions when the actual concentration is near the applicable standard.

To establish noncompliance, one can sample under the assumption that the concentration is equal to the applicable standard. If the count is too light, the concentration is probably in compliance. If the count is too heavy, the standard has been greatly exceeded.

That which influences accuracy and precision of the count likewise influences the accuracy and precision of the concentration measurement. When the count is outside the working (or specified) range, the measured concentration should be tagged with certain qualifiers to help explain or interpret the result.

Suggested reading:

Statistical Aspects

Title — Industrial Hygiene and Toxicology
Volume III: Theory and Rationale of
Industrial Hygiene Practice

Publisher — John Wiley and Sons
A "Wiley Interscience Publication"
Copyright 1979

Authors — Dr. Nelson Leidel (NIOSH/OD)
Ken Busch (NIOSH/DSDTT)

Chapter 3 — Statistical Design and Data Analysis

Concentrate on Section 4, Summarized on page 70

Data interpretation, etc.

Title — Asbestos Analysis- NIOSH Method 7400

Publisher — Applied Industrial Hygiene, Inc.
ACGIH
6500 Glenway Avenue
Building D-7
Cincinnati, Ohio 45211-4438 USA

Author — Dr. Paul Baron (NIOSH/DPSE)

This article is the source of Dr. Paul Baron's quotes given near the end of this module. Dr. Baron, Jay Carter, and Dr. David Taylor were responsible for the revision of P&CAM 239 into Method 7400. This particular article contains a number of clarifications, explanations, and policies relative to Method 7400. A copy of the article is handed out in the course or is included in the course manual.

Statistical Principles

Version #2 Draft 5

April 1988 Stephen G. Bayer, Sr.

This module is part of the instructional materials used in the NIOSH direct-training presentation of the "Sampling and Evaluating Airborne Asbestos Dust Course #582." This handout contains the author's personal viewpoints and has not undergone NIOSH manuscript clearance procedures. Therefore, the content is not intended for use outside the training course and should not be interpreted as universal NIOSH policy. Please do not, in any official capacity, reference nor quote from this manuscript.

objectives:

Describe systematic bias and state two examples

Describe random error and give two examples

Describe the difference between accuracy and precision

Test measured concentration values and determine compliance status

Describe warning and control limits

Given a set of data:

1) Create a frequency distribution
2) Calculate the mean or average
3) Identify the mode
4) Determine the median
5) Calculate the standard deviation
6) Calculate the coefficient of variation
7) Calculate an upper 1-sided 95% confidence limit
8) Calculate a lower 1-sided 95% confidence limit
9) Calculate upper & lower 2-sided 95% confidence limits

Data is a collection of information which represents fact. The word data is pleural. Datum is singular.

Data

The mean or average is the sum of the data divided by the number of data.

Mean

French for "fashion". The mode is the number seen most often (i.e. most fashionable number) in the data.

Mode

When the data is listed in order from lowest to highest, there will be a value which is in the center of the data. For example, if one were to list , in order from youngest to oldest, the ages of everyone in the classroom, the median would be an age that half the class is that age or less and the other half would be that age or older.

Median

When one has a list of numbers, it would be fairly simple to look through the list and find out how many 1's there are, 2's, 3's, etc. This process generates a frequency distribution. Frequency means how often. Distribution depends on how the numbers are classified-- discrete values or group ranges.

Frequency Distribution

When the mean, median, and the mode are the same value (or reasonably close), the data, if plotted as a frequency distribution, assumes a symmetrical, bell-shaped form called a "normal" distribution. The statistical procedures outlined in this module assume a normal distribution and lose validity for other distributions.

Normal Distribution

A measurement that is said to be accurate implies that the measurement is correct. For example, if a student guessed an instructor's height at 6'3" tall, one could say the estimate was accurate if the true instructor's height was 6'2"- providing the 1" error met acceptability criteria! The average is most often used as a "true value" in determinations where exacting standards and analytical methodology is unavailable.

Accuracy

Precision implies reproducibility. The student guessed the height in an accurate manner; however, the estimate might have been a lucky guess! If 10 estimates on 10 different people were equally accurate, then the student's judgement is precise.

Precision

Population

A population is a very large number of individuals, circumstances, objects, or the like that the statistical information is thought to represent. It is very difficult to obtain data on an entire population. Using the asbestos course as an example, a population could be every student who has taken-- and will take-- the course directly from NIOSH. Population does not necessarily mean a group of people.

Sample

A sample is a set of data obtained from a portion of the population. The number of employees, quantity of material on a filter, span of time, etc. should be of sufficient magnitude so as to be representative of the population. For asbestos related professionals, a sample could be data on those students in a given course. A better sample would be information about all who have taken the course over the past year. The word sample can be singular or pleural, depending on viewpoint! Samples taken in one removal project become a sample of removal projects as a whole.

Bias

Systematic bias means inaccuracy "built in" to the procedure or system. The error, or error, can be imbedded into the sampling equipment, procedure, analysis, and/or areas between. The danger of a systematic bias is that its' presence remains undiscovered until comparative determinations are made. A classic example is calibrating a rotameter by using the center of the ball as a reference point. When sampling, someone who adjusts the pump by using the top of the ball will have a systematic error on the high side. A systematic bias can be as subtle as a rounding error in a computer program causing all data to be inaccurate. Another example is a leak between the filter and pump when calibrating. Not all the air goes through the calibrator and the flow rate is inaccurate.

Random Error

When the rotameter ball is being read, two factors come into play. First, five different people would adjust the ball differently-- depending on their vision, opinion of the center of the ball, etc. For each adjustment, the jitter of the ball adds to the uncertainty. These factors cause a certain amount of random error and reduce precision.

Fluctuation of environmental conditions while a sample is being taken and changes in the work process produce some random variability associated with the measured concentration.

Confidence Limit

A confidence limit(s) is a mathematically determined value (or interval between two limits) for which one has some degree of confidence that the true value is between, less than, or greater than. Equations used to determine confidence limits usually look similar in form; however, the "magic numbers" in the equation vary depending on the type of distribution (i.e. normal, lognormal, etc.), what degree of confidence one wishes to establish (often 95%), and whether one wishes to know a range or a greater than or less than value. Sometimes confidence "limits" may be called confidence "intervals".

Practical Example

In the 582 course, the following fiber loadings (in fibers/mm^2) were determined on a given slide by a number of students:

220
337
380
421
433
473
504
581

mean: 419 fib/mm^2

median: 421 fib/mm^2

There is no mode because there are no repetitive numbers. The average and the median are close. The average is generally thought to be the "TRUE" value for asbestos counting because there is no exact way to determine, without human judgement, the correct value! Thus, to determine the degree of accuracy for a given datum, one way is to determine the proximity of the datum to the mean.

The individual who counted 421 fib/mm^2 was very accurate and the one who measured 581 is not very accurate. The fact that eight students arrived at eight different widely-ranging values indicates a fair amount of random variability.

Next is precision. There are three common terms for mathematically expressing precision: standard deviation (S), relative standard deviation (S_r or RSD), and coefficient of variation (CV). The S_r or CV are both easily calculated using the standard deviation. The standard deviation is somewhat laborious to calculate and, for the beginner, difficult to understand.

The eight data were all different; thus, there was variation present in the results. Points of interest are: how much variation is present, how much should be expected under reasonable conditions, are the results within the expected values, and how to report the data in such a fashion that the variability is figured into the concentration value.

For example, any given individual could have (and in fact did) state a singular fiber-loading value. How about this: "The filter has 220 fibers/mm^2!" Another disagrees and says"No! It has 504 fibers/mm^2." Who is correct? Does anyone know? How about "The average fiber density on one slide, measured by eight counters, was 419 fib/mm^2. The counts ranged from 220 to 581 fib/mm^2. Therefore, future counts should not be less than 220 fib/mm^2 nor higher than 581 fibers/mm^2. One might expect most individuals to turn in a count somewhere in the range of 300 - 500 fib/mm^2."

For illustrative purposes, suppose the actual data is unknown, the reader is unfamiliar with the standard deviation, and only two pieces of information are available: average = 419 fib/mm^2 and S = 110 fib/mm^2. In the absence of the original data, these two numbers can be used to calculate ranges and other information.

There is a 95% probability the true value is less than 600:

$$419 + (1.645 \times 110) = 600 \text{ fib/mm}^2$$

There is a 95% probability that the true value is greater than 238:

$$419 - (1.645 \times 110) = 238 \text{ fib/mm}^2$$

There is a 95% probability that the true value is between 203 and 635:

$$419 \pm (1.965 \times 110) = 203 - 635 \text{ fib/mm}^2$$

The concept of probability requires a certain degree of imagination. There are degrees of probability. There is some probability that, anywhere anyone is standing in the entire world, a plane might crash directly upon that spot!. Stand near a busy airport, and the probability is much higher- though still small, indeed. To forecast, one needs to have historical information- DATA!

In the counting example, there are eight counts--six more than are counted on 90% (unsubstantiated seat-of-the-pants estimate by the writer) of the routine asbestos samples counted in all of history!

Predictions are logically made by viewing the facts and circumstances and drawing a conclusion,all things considered. Thus, simply because NONE of the eight counters counted less than 220 or higher than 581 DOES NOT MEAN THAT THERE IS NO POSSIBILITY THAT THE TRUE VALUE COULD BE LESS THAN 220 OR HIGHER THAN 581!

Remember, systematic biases exist. All of these counters could be inexperienced and the true value (determined from an average of 20 experienced counters) is 750. On the other hand, the average of those experts could have been 100 fibers/mm^2 and the high counts resulted from counting wrinkles, dust on top of the cover glass, and cellular debris floating inside eyeballs!

There is then, some small probability that the true value is outside the reported values- especially if the number of data are small! The statistical equations given earlier were used, in the absence of the complete data set, to illustrate or describe, in several ways, the range over which the true value exists- at 95% probability. For now, the topic goes back to what is the standard deviation and how is it calculated?

Again, here is the original data:

220
337
380
421 mean: 419 fib/mm^2
433
473
504
581

Deviation implies that things do not agree. In this data, no one agreed with the average and no one agreed with anyone else. Since the average is the "true value", the lowest person counting deviated 198 less than the mean: the highest, 163 higher. The deviations thus far are -198 and +163.

Deviations

Suppose that one were to calculate the deviations of every datum from the mean. One would have a second column of numbers which are DEVIATIONS. If one were to average that column of numbers, one would have an "average deviation". Such a number would represent the degree of variation of the original data in the same fashion that the mean fiber loading represented the original data.

Before pursuing the calculation process, there is a complication resulting from the nature of a normal distribution. Here are two different plots of the same data grouped differently:

```
150 - 199
200 - 249 *
250 - 299
300 - 349 *
350 - 399 *
400 - 449 *
450 - 499 * *
500 - 549 *
500 - 599 *
600 - 649
```

```
101 - 200
201 - 300 *
301 - 400 * *
401 - 500 * * *
501 - 600 * *
601 - 700
```

A normal distribution is a symmetrical bell-shaped curve when plotted as a frequency distribution. With the exception of needing one more datum on the high side, this data is symmetrical.

Normal Distribution

In an ideal normal distribution there are as many data on the low side as there are on the high side. Therefore, if one were to average all the deviations, the positive deviations would cancel the negative ones and the average deviation would be ZERO! That is the catch-- ONE CANNOT SIMPLY AVERAGE THE DEVIATIONS!

Algebraically, a plus times a plus is a plus and a minus times a minus is a plus. If one were to square the deviation values, one would have a new, third column of numbers-- the DEVIATIONS SQUARED! The numbers would positive, and can be summed and averaged in a meaningful manner.

If one squares a number and then takes the square root, one is back to the original value, Since the deviations were squared to eliminate average values, one could SUM THE DEVIATION SQUARED COLUMN of numbers, divide by the number of data, take the square root, and have a value representing the "average" of the deviations-- the standard deviation based on the actual number of data. What if the number of data is small?.

The standard deviation is a starting point for drawing conclusions for data. The conclusions may describe some reality-- a range of possible concentration values for a specific filter, for example. Another conclusion could be predicting future counts on a reference sample. The accuracy and confidence placed on the predictions and conclusions are highly based upon the number of data.

To build in a safety margin, it is customary to divide the sum of the deviations squared by n-1 data. Again, "n" is a symbol for the number of data. The example in this module has eight counts; therefore, one would divide the third column by seven. Then, take the square root. This standard deviation is then based on n-1 and is almost universally used when one does not have large numbers of data.

Dividing by n-1 inflates the standard deviation value somewhat-- considerably more with a few data and practically none at all with hundreds of data. Since the standard deviation is used to forecast and describe the spread of numbers, any statistical description of the data should be more accurate when using S_{n-1} than when using S_n.

To calculate the standard deviation:

1) determine the mean

2) for each datum- a) subtract the mean from the datum
b) square the resultant value

3) add the squared values

4) divide by n-1

5) take the square root

Congratulations, you now have the standard deviation- $S_{(n-1)}$ hereafter known as "S." The only reason to clarify that point is that calculators often differentiate between the two and that might confuse beginners.

The next step is to calculate the relative standard deviation (S_r or RSD) otherwise known as the coefficient of variation (CV). REMEMBER, the S_r, RSD, and CV are all one and the same-- simple to calculate too. Just divide the standard deviation by the mean!

Statisticians prefer the S_r to be expressed as a decimal value (i.e. $S_r = 0.34$). In everyday conversation, this is usually mentally multiplied by a hundred to get a percentile value-- "The CV is thirty-four percent." Thus one may speak of the S_r as percentile value but, in calculations, always use a decimal value.

Here is a review of the main points. The MEAN or average of a set of data is usually used as the "true value" for the data. Because all measurements do not agree, one must have a means of expressing the variability of the data. One can determine the STANDARD DEVIATION which can be visualized as an "average" deviation value. One can divide the standard deviation by the mean and determine the COEFFICIENT OF VARIATION or RELATIVE STANDARD DEVIATION (S_r or CV) which are useful not only for calculations but also for visualization purposes.

Here is the progress thus far on the original slide data:

Take this #	Subtract av.	Square it
actual data	deviations	deviation^2
220	-199	39601
337	-82	6724
380	-39	1521
421	+2	4
433	+14	196
473	+54	2916
504	+85	7225
581	+162	26244
3349 = Total		84431

n = 8

mean: 418.6 or 419

$$\sqrt{\frac{84431}{7}} = 110 = S$$

$$S_r = CV = \frac{110}{418.6} = 0.263$$

The mean, S, and CV can now be used to interpret the data or to forecast future analyses. Where one is seriously concerned about the presence of significant variability, the mean (alone) is no longer is solely useful for describing the data. A proper description of the data should incorporate the variation. There are three equations which can be used to explain, with 95% confidence the analytical measurements.

Confidence Limits

mean - (1.645 X S)

or

mean - (1.645 X CV X mean)

This equation will predict an upper value-- a value that stands only a 5% chance of being exceeded. Both equations arrive at the same figure. That is because the CV X mean = S. Thus, for the example, there is a 95% probability that the true number is less than:

$419 + (1.645 \times 110) = 591$ fib/mm^2 — 95% UCL, One sided

Such an equation can be used for compliance testing. If the asbestos concentration resulting from 591 fib/mm^2 is less than an air-quality index of interest, then (at this point in time), one could conclude that there is a 95% probability that the concentration is in compliance.

Simply by changing the + sign to -, one can calculate a number lower than the mean. This value would be a LOWER 95% CONFIDENCE LIMIT. Since this equation-- and the last-- calculate a single value on one side of the mean only, the confidence limit is termed "ONE SIDED". If one adds, one determines an UPPER 1-SIDED 95% CONFIDENCE LIMIT. Currently, by subtracting, this is the LOWER 1-SIDED 95% CONFIDENCE LIMIT:

$419 - (1.645 \times 110) = 245$ fib/mm^2 — 95% LCL, One sided

As such, there is a 5% chance the true value for this slide is less than 245 fib/mm^2: however, there is a 95% probability that the true value for this slide is 245 or higher! A concentration resulting from 245 fib/mm^2 would be a number of fibers/cc that would, resulting from this analysis, stand a 95% chance of being exceeded. If that concentration value was in excess of an air-quality standard, one could say that there is a 95% probability that the standard had been exceeded!

These two equations, used separately, calculate one-sided 95% confidence limit values. When used together, the result is different. There is a 90% probability that the true value is between them! Why? In one case there is a 5% chance greater than and, in the other, a 5% chance less than-- and that leaves 90% remaining in between. — Together, 90% between

More common is predicting a range at 95% confidence. Range implies two values, as opposed to greater than and less than. When dealing with a range, one adds and subtracts from the mean:

$$\text{mean} \pm (1.965 \times S)$$

OR

$$\text{mean} \pm (1.965 \times CV \times \text{mean})$$

95% LCL & UCL
2 sided

These equations calculate two values which have the following property: there is a 95% probability that the true value is between them. Since there are two numbers, one on each side of the mean, these equations are called TWO-SIDED 95% CONFIDENCE LIMITS or INTERVALS. For the original data:

$$419 + (1.965 \times 110) = 635$$

$$419 - (1.965 \times 110) = 203$$

Thus, there is a 95% probability that the true number of fibers/mm^2 is between 203 and 635.

Suppose this sample was a reference slide used for quality-control purposes. Using the data from the slide, one could predict that the next person who counts the slide would submit a measurement somewhere between these two numbers. That assumption would be true if the equipment, procedures, student background, and degree of instruction were within specifications. On the other hand, there is a 5% chance that the new measurement might be less than 203 or higher than 635

Control Limits

Using these two numbers as "CONTROL LIMITS" is useful to help identify "outliers"-- unusually low or high counts. Thus, one who was outside of these values could be said to be too high or low. Does this mean a failure?

That depends on how one views the statistic. When one is predicted to be within the control limits 95% of the time, the implication is that one is predicted to be too low 2.5% of the time and likewise too high 2.5% of the time. Thus, one who is outside the control limits more than 5% of the time has a problem.

Warning Limits

Warning limits are commonly assigned as the mean ± S. When one is more or less than 1 standard deviation from the mean, warning limits indicate a tendency for low or high counts. There is approximately a 68% probability that a given analytical value will be within the warning limits.

Control and warning limits may or may not be considered to be "engraved in stone". The mean ± (2.77 S) are typical values for commercial laboratory QC programs. The mean ± (1.965 S) are common for smaller-scale programs.

Another point of confusion on quality control is an equation that is used to test the difference between two measurements to see if the difference exceeds, at a 95% probability level, the predictable difference associated with the average of the two counts. Naturally, the predictable difference will be a function of the variability. Thus, a new, but similar, 95% confidence equation:

Count Bias Test

2.77 X CV X mean = difference not to be exceeded where P = 0.95

P = 0.95 is another way of writing 95% probability. The confusing issue is that three different "magic numbers" have been used to calculate 95% probabilities-- 1.645, 1.965, and 2.77! Using 1.645, adding it calculates an upper 1-sided limit, subtracting it yields a lower 95% 1-sided limit, and adding and subtracting calculates a 90% confidence range or interval. Adding and subtracting 1.965 determined a 95% interval. These numbers and associated confidence values were said to be associated with NORMAL DISTRIBUTIONS

The new element causing 2.77 to be the "magic number" is this: if one were to take a set of data and determine all possible combinations of the absolute (i.e. disregard whether they are positive or negative) differences between any two results, and plot the differences as a frequency distribution, the distribution would no longer have the symmetrical bell-shaped distribution.

Rather, the data would have the shape of half a bell-- with the high point at 0 difference and tapering downward as the differences became greater. There is a module in your course manual which explains the count-testing equation.

In compliance testing, there is another mysterious adaptation of equations described in this module. Using one described earlier:

mean + (1.645 X S) = mean + (1.645 X CV X mean)

The second equivalent equation, using the relative standard deviation (CV), will predict some value that, at 95% probability, would not be exceeded. The problem with routine analyses is this: THERE IS NO MEAN!

No mean?

Nearly every analytical result comes from one sample measured one time by one person. To have a true mean requires multiple data. Should the analyst assume that every measurement is equivalent to the true mean that would exist if, for example, 50 other persons analyzed that sample (assuming that was, in fact, possible)?

Think about an attempt to establish that a given individual, on a given day, is in compliance with a given air-quality standard. The standard is known and, as a result of the analysis, a MEASURED CONCENTRATION is determined. The term "measured concentration" is preferred because the true concentration is unknown. The true concentration can, at best, be a statistically-determined value.

Also known is that variability exists which, in turn, causes an associated amount of uncertainty about the value for a true concentration. Thus, the true concentration is a function of several things-- the measured value, the amount of variability, and the amount of statistical confidence placed on the final result.

The amount of statistical confidence is nearly always 95% from a legal perspective. The amount of variability is a historically-determined value. The measured concentration is a result of the analysis which, one again, is not a true mean because, almost totally without exception, the sample is only analyzed one time- twice at the very most.

Since the measurement is not a true average value, and variability exists, what assumption should be made? Should one assume the measured concentration is a true mean value? To draw this conclusion is somewhat arrogant. What would be better?

In compliance testing, one is comparing a measurement against a standard. The standard is a criterion against which one tests the measurement and determines compliance status. In statistics, there is a rule of thumb for this application It is called the "NULL HYPOTHESIS FOR STATISTICAL TESTING".

Null Hypothesis

The null hypothesis is this: when one is testing a measurement against a criterion, and no mean exists, assume the mean to the the criterion being testing against. In other words, assume the mean to be the air-quality standard against which the measured concentration is being compared. This, too, is an assumption having an error involved. One could also argue that the measured concentration should be closer to a true analytical mean than the air quality standard. Thus, both assumptions have errors involved: there is a difference between the errors.

Assume that the standard is the mean is a "consistent error". The assumption may be incorrect; but, the degree of incorrectness is reliable. If one were to assume the measurement is the mean, the degree of incorrectness would be variable depending on where the measurement fell in the normal distribution of the results-- on the high side sometimes, the low side on others, and, on rare occasion, exactly on the true mean.

Any statistical procedure will benefit the user if the number of data used to draw the conclusion is increased. In compliance testing, n is usually 1! Drawing conclusions from one datum is one of the statistical sins of all sins. Since this is usually the case for asbestos, the one-sided 95% UCL equation, using the Null Hypothesis, becomes:

$$\text{mean} + (1.645 \times \text{CV} \times \text{mean})$$

changes to

$$\text{measurement} + (1.645 \times \text{CV} \times \text{standard})$$

On the left, one uses the measured concentration. On the right, one substitutes the standard against which the measurement is being compared. This particular equation is the one for an employer to use to determine, with 95% confidence, whether or not the measured concentration is in compliance.

Test for Compliance

The customary process for determining compliance status has several possibilities. In order for an employer to prove compliance, the measured concentration must be less than the standard: if the measurement is greater than the standard, the employer concludes that a violation has occurred and no statistical test is necessary.

Violation

Second, if the measurement is less than the standard, the employer could perform the test shown above and, if the measurement was less than the standard but the statistical confidence limit was above the standard,, the employer would conclude possible violation of the standard.

Possible Violation

Third, if both the measurement and the upper 95% one-sided confidence limit were under the standard, the employer could conclude compliance status.

Compliance

Remember, the employer may need to prove, with 95% confidence, that the concentration in question did not exceed the standard. An adversary, asserting that the standard was exceeded, must prove-- with 95% confidence-- that a violation occurred. To accomplish such the latter conclusion, the following equation is used:

Test for non-compliance

measurement - (1.645 X CV X standard)

This is a regulatory agency's test for determining compliance status. The basic principles just described are not guaranteed to be policy for regulatory agencies. When in doubt about policy, ask the appropriate agency. Once again, under the assumption that one sample has been taken, the null hypothesis is applied: substitute the standard for the measurement. The conclusion on compliance status is similar but inverted.

If a compliance officer has a measurement which is less than the standard, the status is compliance.

Compliance

Possible Violation

If the measurement is greater than the standard but the lower 95% confidence limit is less than the standard, violation is a possibility-- but so is compliance.

Violation

To conclude violation of the standard, both the measured concentration and the 95% lower confidence limit must be in excess of the standard.

The reader may now see that there will be a "gray" area between a concentration that the employer can establish compliance and the regulatory agency can establish non-compliance. This gray area is a function of two factors-- the variability (i.e. CV) and the null hypothesis.

Standard Error

If more than one consecutive sample is taken, the Null hypothesis complaince testing equations can be modified by dividing the S_r X Standard (AQS) by the square root of the number (n) of consecutive samples taken. Dividing by the number of samples taken reduces the uncertainty present (resulting from not having a true average value).

The "standard error" approach to the equation applies to a circumstance where one takes several immediately-sequential samples (or, especially, simultaneous) within a population that is uniform. An excellent example would be simultaneous environmental samples inside the NIOSH classroom.

Representation is a key consideration. If all the samples are not taken in such a fashion that all represent the same population, one should not use the "standard error" in the equation.

$$\text{Standard Error} = \frac{S_r \text{ X AQS}}{\sqrt{n}}$$

Thus, the compliance-testing equations now become:

Measured Concentration (+ or -) 1.645 X SE

SUMMARY OF EQUATIONS

$$\text{Average count} = \frac{\text{total fibers seen}}{\text{total fields observed}} = \frac{\text{fibers}}{\text{field}}$$

$$\text{Fiber density} = \frac{\text{average count fib/mm}^2}{\text{field area mm}^2\text{/fld}} = \frac{\text{fibers}}{\text{mm}^2}$$

Standard deviation =
1) for each datum, subtract average, then square it
2) sum the resultant values
3) divide by n-1
4) take the square root

$$S_r \text{ or } CV = \frac{\text{standard deviation}}{\text{mean}}$$

$$\text{Standard error} = \frac{S_r \text{ X Standard}}{\sqrt{n}} = SE$$

Test for COMPLIANCE = measured conc. + 1.645 X SE

Test for NON-COMPLIANCE = measured concentration - 1.645 X SE

For a single sample, the equations also appear in the following format:

measured concentration (+ or -) 1.645 X S_r X standard

Compliance-related decisions should not be based on one sample.

Employer's Viewpoint

For an employer to be in compliance, neither the measurement nor the value in the test-for-compliance test can exceed the standard. If the measurement exceeds the standard, the employer considers the circumstance a violation. If the measurement is less than the standard but the test-for-compliance value exceeds the standard, the concentration should be regarded as a possible violation.

Regulatory Agency's Viewpoint

To establish a violation, neither the measurement nor the test-for-noncompliance value can be less than the standard. If the measured concentration is less than the standard, one concludes compliance status. If the measurement is greater than the standard but the statistical test value is less than the standard, violation is only a possibility.

Note

These tests for compliance use the Null hypothesis for testing a measurement (which is not a true average value) against an air-quality standard which applies to the purposes for sampling. The equations estimate compliance status at 95% probability (i.e. P = 0.95).

The following equations are applied to data from which a true mean is available. A typical example would be counts from reference slides in a quality-control program.

1) There is a 95% probability that the data is less than:

   mean + (1.645 X S) — 95% UCL 1-sided

2) There is a 95% probability the data is greater than:

   mean - (1.645 X S) — 95% LCL 1-sided

3) There is a 95% probability the data is between:

   mean $\pm$ (1.965 X S) — 95% UCL & LCL 2-sided

4) There is a 95% probability the difference between counts does not exceed

   2.77 X CV X mean — 95% Bias Test for Counts

NOTE: CV X mean can be substituted, where desired, for S: S = CV X mean

In equation 4, the mean is usually based on the average between the two counts in question.

Statistical Worksheet

NOTE: u will represent mean

x Data	x-u Deviations	$(x-u)^2$ Deviations^2

Sum of data: _____ Sum of $(x-u)^2$ = _____

(mean) u : _____ $\frac{\text{Sum of } (x-u)^2}{n-1}$ = _____

$S = \sqrt{\frac{\text{Sum of } (x-u)^2}{n-1}}$ = _____

S_r or CV = S/u = _____

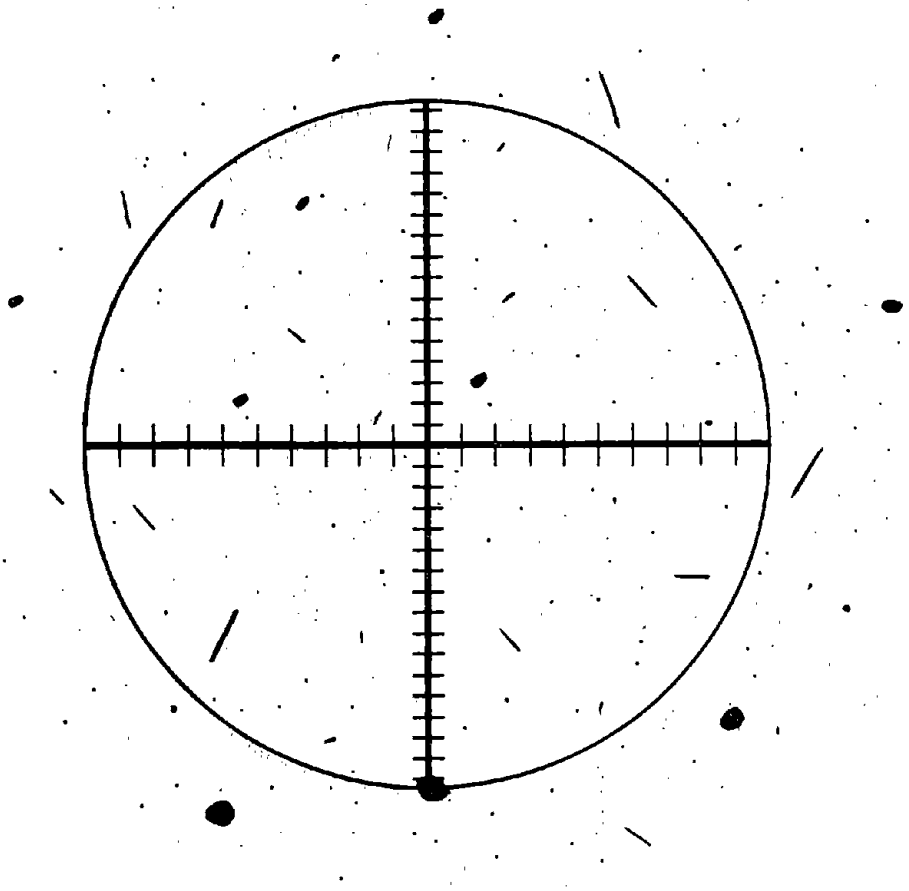
P = 0.95 true value is less than..... u + (1.645 X S) = _____

P = 0.95 true value is greater than..... u - (1.645 X S) = _____

P = 0.95 true value is between..... u $\pm$ (1.965 X S) = _____ - _____

Note: S_r X u can be substituted for S

B



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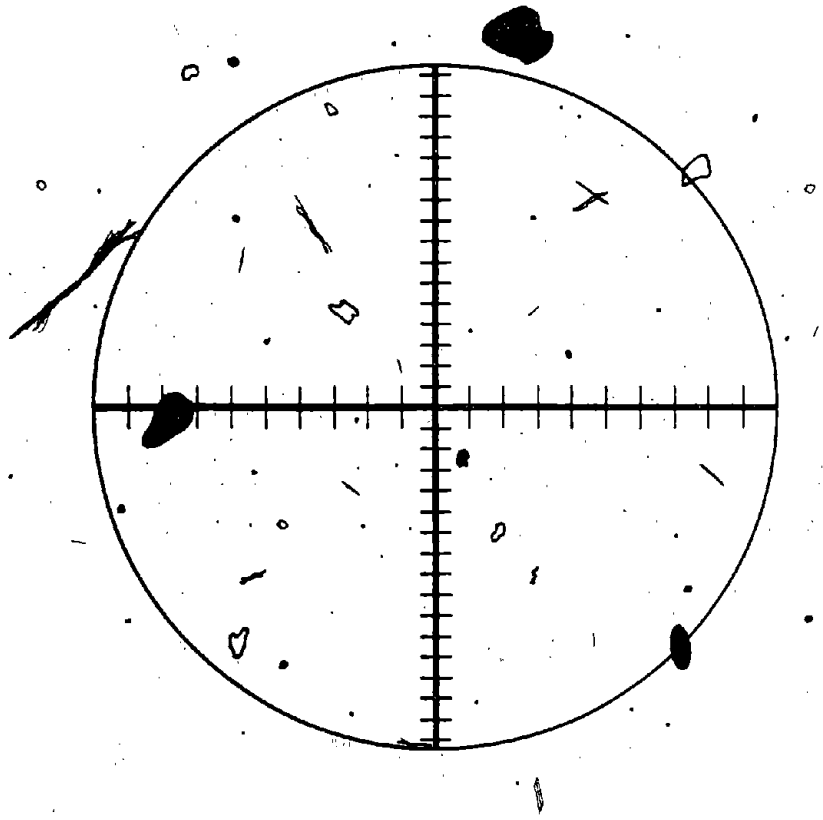

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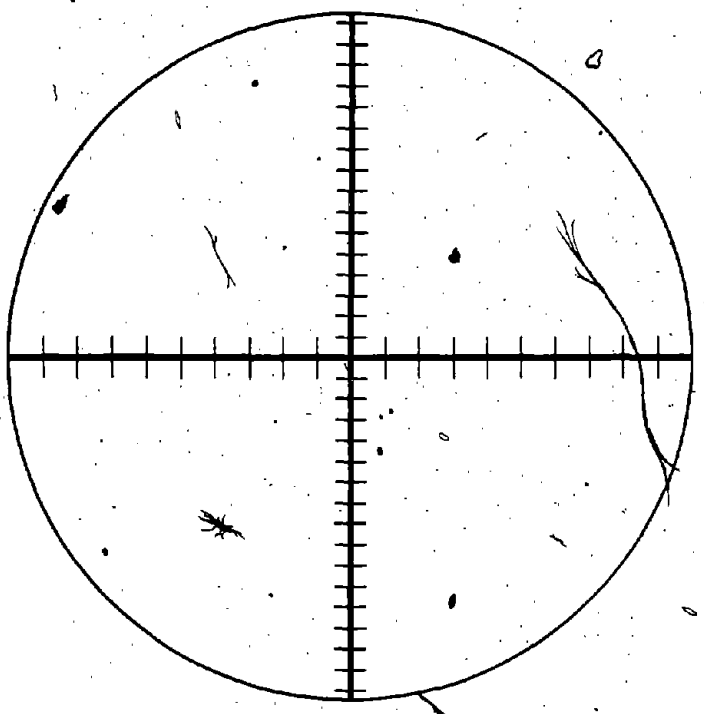

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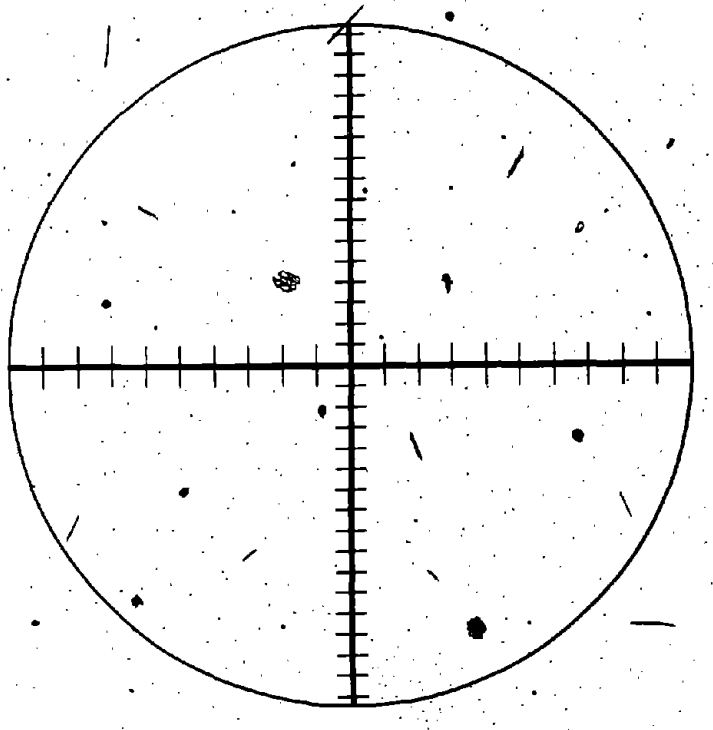

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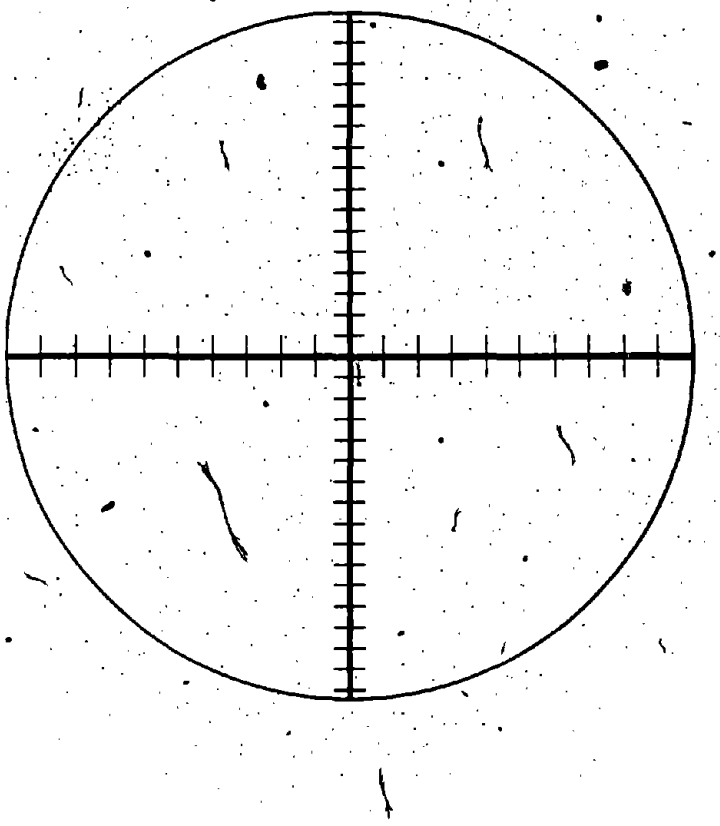

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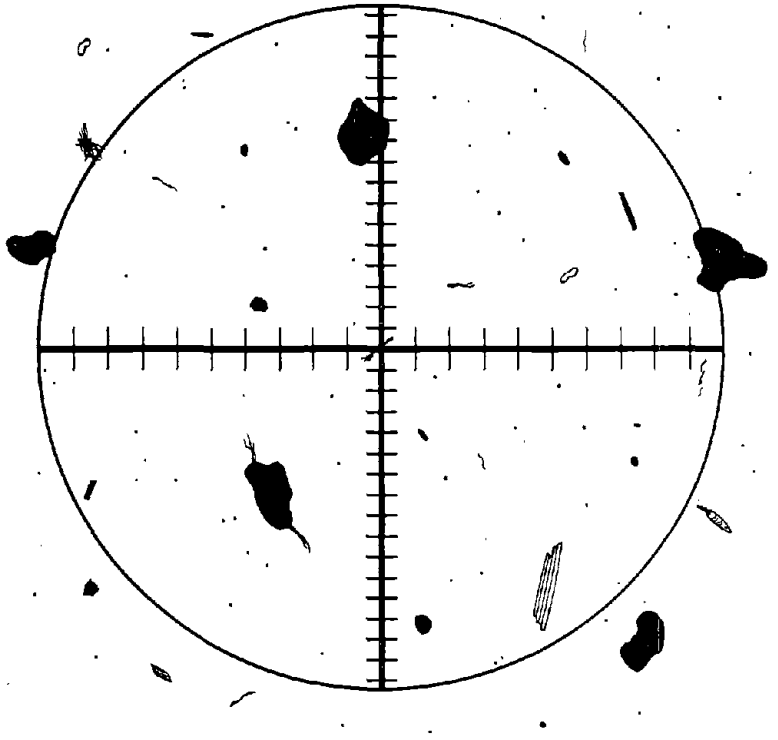

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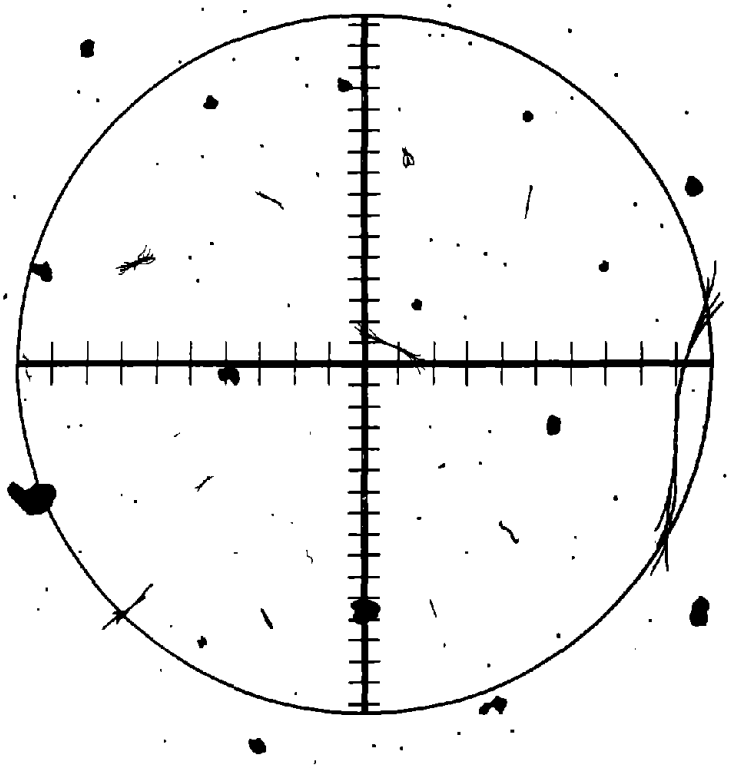

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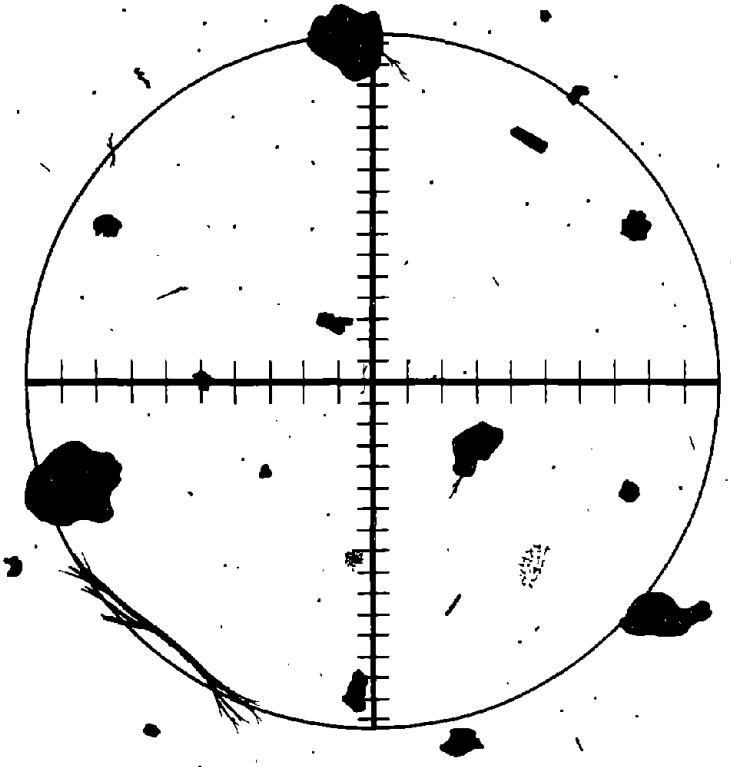

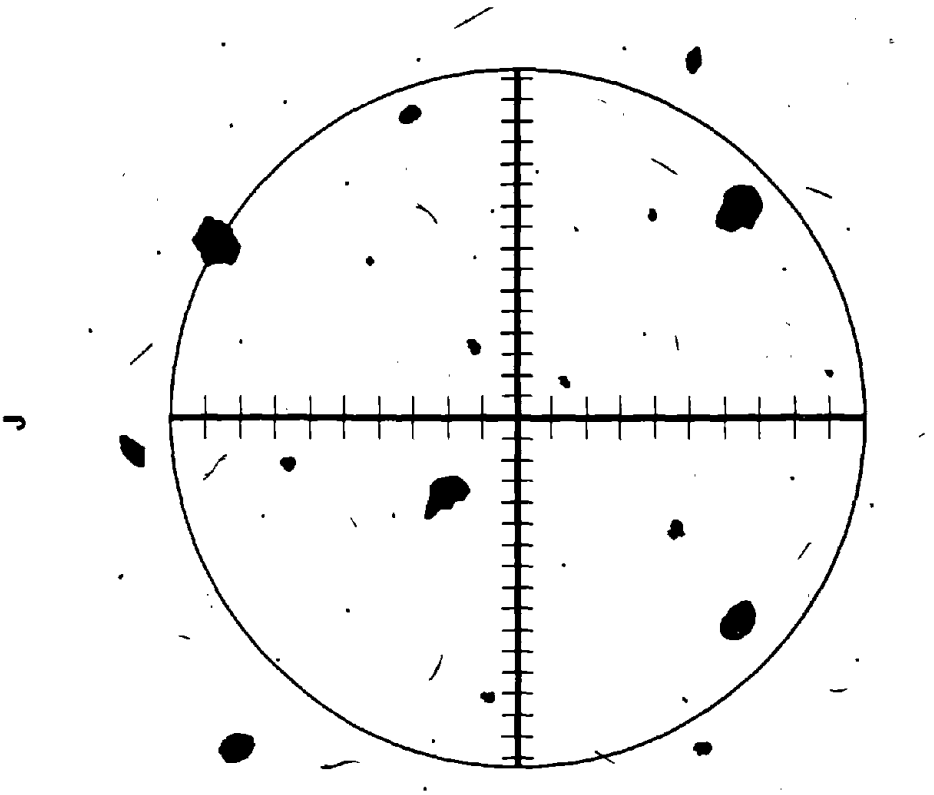

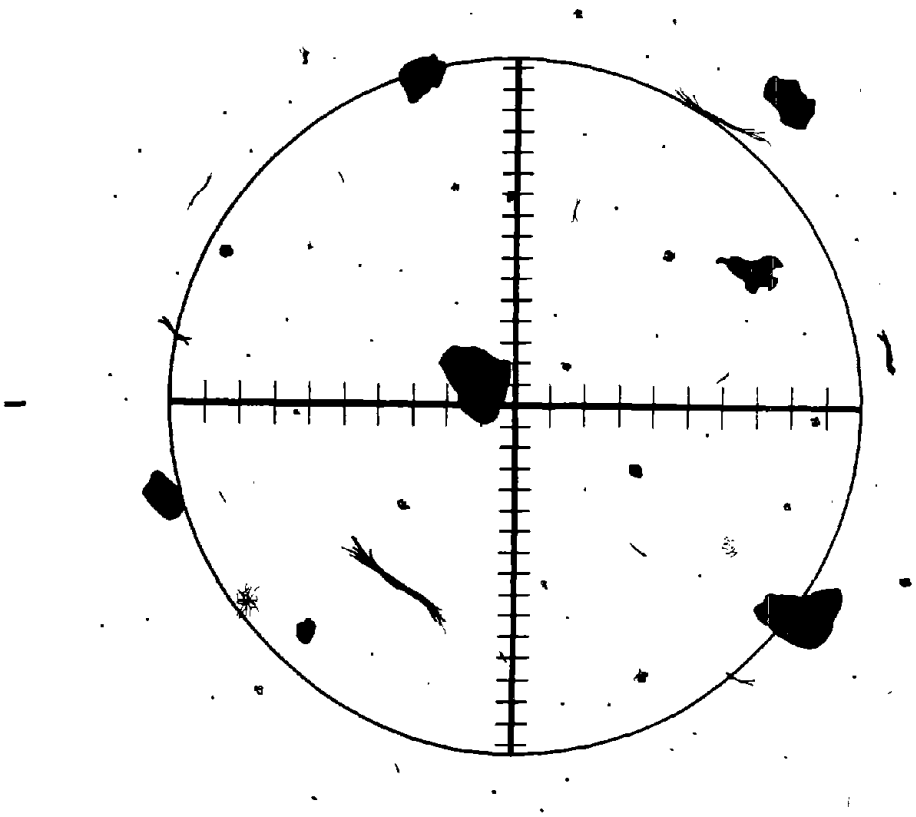

Familiarization with the Microscope

April, 1988

Stephen G. Bayer, Sr.

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Please do not, in any official capacity, quote from nor reference this document. Please refer to NIOSH Method 7400 and read the sections on equipment specifications and the section on microscope quality control.

Mention of commercial concerns does not constitute endorsement by NIOSH.

Objectives:

1) Identify the components of the microscope

2) List the three basic steps to the setup procedure

3) List necessary accessories

4) State any specific specification or design criteria

Students will be expected to know all major microscope components by name and function (purpose).

Students will be held responsible for stating any major equipment specification (i.e. Kohler illumination, Walton-Beckett graticule), adjustment principles (how to adjust the field iris), quality-control procedure (log book information, resolution/contrast inspections) or major design characteristic (numerical aperture range, overall magnification) if such information is covered in Method 7400.

Students will not be expected to describe theories and concepts not covered in the method nor to explain, for example, how phase contrast works, why a condenser is needed, or define phase relationships.

Components

Modern microscopes are selected basically by model. In general, the ranking (from relatively bad to excellent) follows this approximate scheme:

1) Student (budget)

2) Laboratory (scientific)

3) Research (laboratory grade, but more versatile)

4) Universal research (high $, high versatility)

One occasionally hears of polarizing or petrographic microscopes. A petrographic microscope is usually a polarizing microscope with additional versatility or capability. Petrography is a science related to using a microscope to classify rocks and minerals. A polarizing microscope would be useful for a chemist, for example, and may not need the additional capabilities required by a petrographer.

A polarizing microscope can be converted into a phase by simply purchasing a phase contrast objective and condenser. The objective lens is usually semi-permanently screwed into the nosepiece and the condenser is mounted into place when needed (about ten seconds time, usually involving loosening one screw at most). A polarizing microscope requires several additional components (notably a rotating stage) which can be costly and inconvenient when trying to convert a phase-contrast microscope into a polarizing model.

Most phase-contrast microscopes are in the laboratory category. Qualities that most prefer, when purchasing a phase-contrast microscope, include the following:

1) Very little free play in adjustments

2) Illumination of adequate brightness (with filter)

3) Comfortable height (some are taller than others)

4) Centerable optics (including light bulb)

A basic microscope has three basic parts:

1) The stand

2) The magnifying components

3) The illumination system

The stand is, for the most part, the microscope itself, as seen from a distance. The stand incorporates a foundation on or in which the remaining components are mounted. The base of the stand contains the illuminator. The head is at the top below which the objectives are mounted (in a nose piece) and above which is mounted the body tube (typically binocular) containing two eyepieces. Between the illuminator and the nosepiece is a platform called the stage. Beneath the stage is a mount for the condenser.

The magnifying components include (in order as though looking downward through the system) the eyepieces (or oculars), the body tube (including the binocular prisms, optical pathways, and nosepiece), and the objective lens.

The illumination system (from the bulb to the slide) includes a light bulb, projecting lenses, mirror, field iris, and condenser.

The method states that Kohler illumination is is preferred. Kohler illumination specifies three basic requirements: Illumination

1) The specimen is in focus

2) An image of the field iris is in focus at the specimen plane and its edges are adjusted and nicely centered on the edge of the field of view (everything seen through the microscope)

3) An image of the light bulb filament is in focus at the plane of the condenser iris.

The reason the method states that Kohler illumination is preferred is that few microscopes have true Kohler illumination (usually lacking capabilities for accomplishing the third criterion listed above).

Focus filament

The purpose of Kohler illumination is to provide a crisp beam of illumination. Contrast assists visibility. A low contrast image could be as visible as a high contrast image; however, subtle detail in the image might not be as noticeable. There are times when contrast is wanted: looking for very fine fibers (which tend to be light gray) against a medium gray background is one time contrast is helpful. Contrast helps sharpen the edges of imaged objects helping to detect the objects against the background.

For reasons too lengthy to cover here, most microscopes have tiny light bulbs with ground glass placed in front of them. The ground glass diffuses the light which, in turn, softens the illumination beam somewhat. Thus, this requirement (or capability) of Kohler illumination is absent in most modern laboratory-grade phase-contrast microscopes.

If one could focus the filament, an ideal filament would be a solid strip of luminous metal which would be projected by lenses, bounced off a mirror and directed onto the condenser. The lenses would be moveable (or the bulb) in some fashion such that the surface of the enlarged filament would fill, be in focus at, and centered nicely across the plane of the condenser iris.

Microscopically speaking, an iris is a set of overlapping blades of metal or plastic. The blades couple into an adjusting collar or lever which, when moved, twists on the blades. The blades overlap to form an opening through which light shines. If six blades are used in the iris, the opening would be hexagonal. With eight or more blades, the opening becomes increasingly circular.

The condenser iris is used for bright-field microscopy (ordinary microscope, in other words) and is either not in place or completely open for phase-contrast microscopy. Thus, to the newcomer, condenser iris is either a mysterious item of no use or is a term confused with field iris (which is an opening in the illuminator).

Thus, if one could could look underneath the condenser and see a focussed image of a coil-filament bulb, one would see a one-dimensional zig-zag representation (image) of the coil sharply in focus, filling the whole condenser opening, and nicely centered.

Focus on Sample

Before the field iris can be adjusted (Item 2), one must be in focus on the sample. Typically, this is the first order of business since adjusting the light bulb is either impossible, or the bulb needs replacing (in which case one follows manufacturer's directions). Focussing on the sample is about as straight forward as the statement sounds. When the sample is in focus, the coarse and fine focus knobs are not readjusted until the following step(s) are completed.

Adjust Field Iris

The field iris is an adjustable opening in or near the base of the microscope. Some microscopes (typically student models) do not have field irises. This means that one is as far away from the method's illumination recommendations as one can get.

To help Kohler illumination provide contrast to the image (that is the purpose, after all), the field iris performs two purposes. It places the focal point of the condenser directly upon the plane of the sample (which is still in focus) and the field iris helps to eliminate scattered light which causes glare.

A slide (audio-visual transparency type) projected on a screen in a typically lit classroom will be hard to see. Why? The ambient light fills in darkened areas and reduces the contrast of the image. See? Contrast equates with visibility. Turn out the room lights and the image is easier to see even though the projected image is <u>not</u> as bright as before.

When you are looking at a sample, you are looking at a very tiny area. If the field iris is nonexistent, or opened all the way, the sample is flooded with a wide beam of light. The objective is collecting light coming from the area defined as the field of view. Some of the light outside the field of view can be scattered into the field of view and, similar to an AV slide in a bright room, the image is, to some degree, washed out.

Thus, one major purpose of the field iris is to provide a barrier to the illumination beam such that the only portion of the slide that is lit is the tiny area you see through the microscope.

Next, the field iris finds the condenser's point of focus. The beam of light coming out of the base of the microscope is very large compared to the extremely tiny field of view.

The purpose of the condenser is to provide oblique illumination. Think of each photon of light as a piece of data. The more directions one could pass those little photons through a sample and, in turn, collect them into the objective, the better. Each photon carries information about some aspect of the material under observation.

If you were to examine a statue. If one could only see the statue from one point of observation, one's interpretation would be limited. Moving around and observing the statue from many different angles would provide better information about the statue as a whole.

Thus, the purpose of a condenser is not to make the light bright: a bigger bulb would do that. The condenser brings light to the specimen in a variety of directions, each carrying a different perspective with slightly more information. The final result is an improvement in resolution.

To get these photons to converge on the field of view, the focal point of the condenser must be right on the specimen.

Adjust Field Iris

To adjust the field iris, one performs several minor tasks. Given is that one is actually looking at the specimen through the microscope.The tasks are:

1) Close the field iris

2) Focus the field iris

3) Open the iris nearly to the edge of the field of view

4) Center the iris if needed.

5) Adjust the field iris diameter to exactly coincide with the edge of you field of view.

In quick retrospective summary, the field iris was focussed to place the condenser at the point of focus (on the sample) and the blades now block out stray light and illuminate only what is seen through the microscope.

The condenser provides a focussed cone of illumination which passes at a variety of directions through the material in the field of view.

If the light bulb filament was focussed at the plane of the condenser iris, this microscope would now have been adjusted for observation under Kohler illumination.

Kohler illumination, at this point, is irrelevant to phase contrast. Kohler illumination is simply a way to illuminate the sample for maximum resolution and contrast. Phase contrast takes the contrast-producing process one giant step further.

Numerical Aperture

The condenser provides a cone that converges onto the sample which, in turn, diverges and is then collected by the objective. The angle of convergence/divergence is represented by a number termed the numerical aperture. The larger the value, the wider (greater) the angle. The higher the N.A., the more directions light passes through the sample which, in theory, provides increased resolution. The numerical aperture of the condenser and objective would be exactly the same for a matched pair. This, however, would limit the use of that condenser to objectives having identical N.A.'s. To make systems more versatile, condensers often include flip-up lenses which can reduce the condenser's N.A. for lower-powered objectives (which typically have lower N.A.'s).

The N.A. of the 40 - 45X objective specified in the method is given as 0.65 - 0.75. A condenser must have an N.A. <u>equal to or greater than</u> that of the objective. The N.A. is almost always stamped on the objective and condenser. Typically, one could expect to see 0.65 to 0.75 somewhere on the objective and 0.9 on the condenser.

Phase Contrast is considered a form of illumination. It is a way of altering the nature of the light that passes through the field of view. A common bright-field microscope depends on differences, in refractive index, between the material being observed and the medium in which the material is suspended.

Refractive index is a number starting at 1.000 representing optical density or degree of resistance to light passing through a given material. The world of refractive index values range from 1.000 to 3.000. Low refractive indices are around 1: water, for example is around 1.333. Optical glass, in your microscope, is typically 1.515. Amosite asbestos is around 2.77-- fairly high.

Not only do differences in refractive index play a part in visibility, the thickness of an object also plays a role. The smaller an object, the more difficult it is to see an object near in refractive index to the medium.

Phase Contrast is used to see objects which are close, in refractive index, to the medium. Phase contrast uses phase differences between the light which passes through a fiber (for example) as compared to the beams of light which pass by the edges of the fiber.

A phase-contrast microscope has two basic components: a dark ring in the objective lens and a transparent (bright when the illumination beam passes through) ring in the condenser.

Typically, there is a unique transparent ring in the condenser (called an annular diaphragm) for each particular objective (containing the dark ring called a phase-shifting element). If one could remove the eyepiece and look down through the optical system, one would see two rings- the bright annular diaphragm and the dark phase-shifting element. If the system is properly selected and adjusted, both rings would be the same size and perfectly superimposed or concentric. In other words, the rings would be analogous to two donuts which are the same size. One could stack one on top of the other and, when looking downward, adjust them such that only one donut would be seen!

Thus, for any particular objective, there is an appropriate annular diaphragm. The phase-shifting element (dark ring) is permanently mounted in the objective lens. The annular diaphragm is either mounted in a turret inside the condenser or is available as an insert component. The alignment process is performed by screws or knobs (on the condenser) which move the annular diaphragm until concentricity is achieved.

For phase contrast to work properly, the rings must be as concentric as possible. The light from the annular diaphragm (bright ring) must pass through (and be reduced in intensity by) the phase-shifting element (dark ring).

There are a number of ways to design phase-contrast optics depending on the degree of contrast needed and bright on dark fibers or vice-versa. The type specified in the method is termed "positive contrast" which has a neutral (gray) background where the fine fibers are dark and generally become bright as they become thicker.

To inspect the quality of the image, meaning the overall combination of contrast and resolution, the method requires periodical inspection of the image using the HSE/NPL Mark II test slide.

The test slide consists of two coarse (a relative term; they are really fine lines) sets of horizontal lines and two sets of horizontal lines. These lines serve as "finder" lines for the region in which the resolution/contrast (test) lines are viewed. The test lines are not visibly numbered; but, come in a series of seven sets of about twenty lines (or grooves). The first set is on one end of the rectangular area defined by the finder lines and will be fairly easy to see at 400X unless the microscope is in extreme misalignment and grossly dirty.

Focus on the first set and, as will be summarized later, adjust the microscope to counting status. Look for the second set of test lines. They are next to the first set. Continue this process, mentally keeping track of how many sets you see.

The method says you must see the first three completely. The fourth or fifth must be partially visible. If the fourth set is partially visible, the fifth set cannot be seen! Partially visible means that the lines do not have continuity. One sees them in some places but not in others.

The method states that the sixth and seventh set must be invisible. The purpose of the test slide is to provide an element of consistency in imagery thereby increasing the overall national precision (i.e. reducing variability).

Generally, most microscopes have turret-type condensers. One twists the perimeter of the condenser and, relative to indicating mark or window, markings click into place. The markings may be a numerical scheme (i.e. 1, 2, 3, etc.) or marked by objective power (i.e. 10, 20, 40, or 20/40, etc.) The correct annular diaphragm must be in place. If both rings, as seen through the optical system, can be superimposed one atop the other, the proper rings have been selected regardless of the markings.

Telescope

The rings are easy to see in a few microscopes by simply removing the eyepiece and looking into the internal workings of the optics. Typically, however, the rings appear very tiny almost as though they are far away. To assist in inspecting the centering, one needs a small telescope which momentarily replaces the eyepiece. The telescope is focussed by holding the lower part with two fingers and then pulling (for friction fit) or twisting (for helical threads) the eyelens. Quickly, the rings will come into focus and the centering can be performed.

Centering

Centering is accomplished in a variety of ways. In nearly all microscopes there are screws, sliders, or knobs which one manipulates to move the annular diaphragm (bright ring). Typically, the controls (found on the condenser, naturally) are permanently mounted and are near the front of the condenser or perhaps mounted near the sides. Some microscopes have detachable "wrenches" that are inserted into hole and, in turn, engage centering screws. One is not advised to purchase "precentered" systems which supposedly never need adjustment. What if they <u>do</u> need to be adjusted?

Pause for a moment on that basic question. The author's basic experience with people finds two basic technical classifications: the chronic knob twisters and the on-off button types. There are pro's and con's for each preference. The author tends to prefer the following: the more adjustments, the better. A misaligned component that cannot be corrected presents a frustrating circumstance. Frustrating for others is the process of learning what the adjustable components are and how to perform the adjustments.

Head

There are many options on what kind of head or body tube to buy. Typically, most prefer a binocular head which contains two eyepieces and permits a normal viewing condition. If most didn't prefer this viewing environment, why don't people take telescopes to sporting events?

For binocular heads, there are basically two types: true binocular forms and fixed binocular forms. The fixed version has two prisms mounted in a fixed position and the eyepieces slide inward and outward (relative to the position of the nose when viewing through the scope), but the head does not move. The minor difficulty with this type is that the distance from the objective to the eyepieces is further in the widest position than the distance is in the narrowest position. The objective-to-ocular distance is termed the tube length.

A standard tube length will allow one to multiply the objective power (i.e. 40X) by the ocular or eyepiece power (i.e. 10X) to determine the overall magnification (i.e. 400X) Fixed binocular heads can produce a slight shift in magnification (i.e. calibration of field area, etc.), depending on the setting of the interpupillary distance (the distance between the eyes). Normally, this calibration shift is negligible and, in some cases, there are other adjustments which permit compensation. Unless two people having opposite extremes in interpupillary distance are using the same scope, there is seldom a significate problem.

True binocular heads (more expensive, naturally) have prisms which move in an independent fashion maintaining a constant tube length regardless of interpupillary distance.

Oculars

Eyepieces, or oculars, are varied in design. For any given magnification, there are many ways to design lens systems for magnifying the image coming from the objective. Generally, most buy wide-field eyepieces. The images compare to the difference between looking through the paper tube from a roll of paper towels as compared to looking through a cheerleader's megaphone. That which you see is a wider field of vision through the megaphone and through wide-field oculars.

Also consider buying focussing eyepieces which assist in focussing on the graticule (mounted inside one of the eyepieces and soon to be discussed). One or more of the ocular's lenses are (or should be) in focus on the graticule. The ocular, as a whole, is in focus on the image coming from the objective lens. If both are in correct focus, a well-focussed image of the sample and a well focussed graticule image will appear. If the graticule is out of focus, a non-focussing eyepiece can usually (<u>not</u> always) be corrected by unscrewing the top lens. If the eyepiece is focussable, the ocular components are adjustable by friction or by threads.

For the most part, the microscope corrects for near and far-sightedness simply by using the coarse and fine focus knobs mounted on the stand. The microscope cannot correct for astigmatism. Astigmatism is a non-linear directional error associated with the shape of the eye. If one has astigmatism, a line drawn from north to south, for example, will appear sharper than an identical line drawn from the northwest to the southeast. If one wears glasses to correct astigmatism and then removes the glasses to see through the microscope, fibers (at the limit of visibility) resting in one orientation may be seen while identical fibers other orientations may not be seen.

To correct this problem, there are eyepieces designated as high-eyepoint. Such an ocular projects the image higher and allows room for glasses (between the eyeball and the ocular).

There are a lot of names and terms for eyepieces. Examples are Huyghenian or Huyghens, Ramsdens, Periplans, Hyperplanes, and others. Typically, you can ask for a wide-field focussing ocular and, if needed, high eyepoint also.

Graticules

A graticule is generally considered to be an image which is typically deposited on a disc of glass and is inserted into the eyepiece or ocular. As mentioned earlier, the ocular is in focus both on the image coming from the objective lens and the graticule rendering both in sharp focus.

Historically, for asbestos-counting purposes, the most common graticules were the Porton and the Patterson Globe and Circle. These were procured "off the shelf" and installed in the microscope. Various dimensions (field areas, circle sizes, etc.) were not uniform resulting from variations in optical systems (type of oculars, overall power, tube length, interpupillary distances, etc.)

The result was that rarely did any particular circle coincide to 5.0 um and the field areas (of Portons) ranged from 0.003 mm^2 to 0.01 mm^2, averaging at about 0.006 mm^2. This variation naturally has an effect on analytical variability and accuracy bias.

Method 7400 standardized on the Walton-Beckett graticule which is of two types: G-22 and G-24. The G-22 has fibers drawn outside the field area which are drawn in the proper (A rules) aspect ratio. The B rules use 5:1 aspect ratio corresponding to the fiber aspect ratios on the G-24.

By design, a Walton-Beckett is a custom-order graticule. Even if one comes with a new microscope, someone custom-ordered the graticule. Details on ordering and calibration are covered later in the course.

For now, the important thing is the Walton-Beckett should have, under magnification in counting status, a field area diameter of 0.01 mm (i.e. 100 um). If so, the course hashmarks will be 5 um apart.

Why use a Walton-Beckett? Field area standardition, accurate 5 um (± 2%) size comparator, and comparators for aspect-ratio estimations. To the best of the author's recollections, no other graticule was previously designed specifically for asbestos counting.

Stage Micrometer

To calibrate the field area, etc. one needs a stage micrometer which is nothing more than ruler, so small that it is mounted on a microscope slide and the divisions can only be seen under magnification. Instead of feet, inches, centimeters, or millimeters, the finest divisions on the stage micrometer should be 0.01 mm!

Filters

The method also recommends a blue or green filters. A phase-contrast microscope uses differences in phase between the light that goes through an object (i.e. asbestos fiber) and the light that passes by the edges of an object. If the object and the mounting medium are exactly the same, there is no difference between the light that goes through and the light going around and the object is simply invisible. If the object is different from the liquid, the light passing through the object passes through faster or slower than the light passing around.

Phase is a term describing how two things are travelling along together. Since light is a waveform phenomenon, in phase means two beams are travelling up and down together. Out of phase means one is moving up while the other is moving down.

If the in-phase beams are combined they interfere with one another producing a beam of light brighter than expected from the two beams. If they are out of phase, they cancel one another out, and the result is a darkened beam of light.

Since the general background of a filter is (on the average) the same everywhere, the random nature of vibrating light beams will render the image neutral gray (in white light).

A fiber resting on the background will now pass and scatter (diffract) some light passing through and (in very close proximity) by. There will be a small phase difference between the light going through and the light passing the edges nearby. The scattered light moves out, away from the original ring of condensed light produced by the annular diaphragm. Since the fiber is expected to be higher in refractive index than the mounting medium, the fiber holds the light passing though back in time.

This means that, on the average, the light going through the fiber is retarded in phase (not in phase) with the light passing the edges of the fiber.

Recall that out-of-phase light beams interfere in a destructive manner, producing an overall reduction in brightness? There are still a few problems to solve. First, much more light is going through the fiber as compared to the small amount diffracted (scattered) by the edges of the fiber. Second, the fiber only held back the light for a very minute amount of time which amounts to a very slight phase shift- probably not enough to matter. Third, long red wavelengths do not interfere very well with short blue wavelengths.

Problems

As expected, the problems were solved. The dark ring in the phase-shifting element reduces the intensity of the light that passes through the fiber (i.e. contained in the bright ring). Typically, on or under the dark ring, there is a thin calibrated layer of transparent phase-shifting material which acts as a phase difference amplifier.

The beam that went through the object is now similar in intensity to the light that went around and, for fine objects, more out of phase. The numbers of photons to interfere are more equal than before and more out of phase: not destructive interference can occur producing dark fibers against a gray background. Very minute differences in phase (invisible to the eye) are converted to amplitude differences visible to the eye as brightness differences. By using a narrow-band green filter, the light waves are more similar in length, producing enhanced interference.

Most phase-shifting elements (if not all) are calibrated for 1/4 wavelength phase shift of green light. That is why a green filter should enhance contrast. "Blue" filters (typically supplied) are not really blue. They are "bluish" and are included for the purposes of "warming up" the slightly-red light color produced from the light bulb.

For routine observation, there are three routine steps to be performed before counting each and every slide. The second and third step may not need to be performed; but, they are to be inspected. The reasons for performing these steps were outlined in the sections on phase-contrast theory and Kohler illumination. The steps are:

1) Focus on the sample

2) Adjust the field iris

3) Center the phase rings

Naturally, like all the vast minute procedures we take for granted when driving a car, there are many small steps involved in operating and adjusting a microscope. The three steps described above represent the major operations. General additional considerations are:

A) Illumination at the proper level?

B) Correct interpupillary distance?

C) See sharply through both eyes?

D) Annular diaphragm evenly illuminated?

E) Graticule in sharp focus?

Most of these points have either been covered in this module or can be performed by following manufacturer's literature.

Remember, adjusting the field iris assumes that the sample is in focus and that you are looking through the microscope at the sample. Adjust the field iris by:

Adjusting the field iris

Close the field iris. You are looking for a well-defined "spot" of light against a black background. A fuzzy blob of light or a donut or complete blackness (which may also be a far-off-center iris) indicates that the field iris is out of focus.

To focus the field iris, turn the condenser-focus knob. The knob is underneath the stage and moves the condenser up and down. Notice that when the iris is in focus, the condenser's top lens nearly touches the slide.

When the iris is in focus, you will see a hexagon, if the iris has six blades, or a more circular spot of light if the iris has eight or more. Slight color fringing is normal: the colors come from the chromatic aberration present in the condenser lens glass.

If, as the field iris is opened toward the edge of the field of view, the opening is off center, there are usually centering controls somewhere. Typically, they are found somewhere around the condenser mount. Zeiss has two such controls hidden near the back of the stage. Nikon has two knobs on the condenser mount. DO NOT confuse these with the phase-ring centering knobs usually mounted on the bottom of the front of the condenser.

When the iris looks centered, open it to a diameter equal to the microscope's field of view.

Thus, the major operations involve focussing and centering the field iris. The final goal is that the blades of the iris should be as crisp as possible and that, when the iris is opened, the iris will exactly coincide with the perimeter of the field of view. These sub-steps usually require less than eight seconds of time and centering is typically not required. The focus will usually change slightly on a slide-to-slide basis.

Center phase rings

Remove one eyepiece and insert the centering telescope.

Using two hands, push-pull the components or twist the top lens for threaded mounts. Focus the telescope on the phase rings. If the sample has a moderate amount of material present, the scattered light will "light up" the inside of the objective and the dark ring is easy to see against the bright ring. If the sample is light, the dark ring is difficult to see against the black background.

Find the two knobs or sliders. The Nikon microscopes have two knobs hanging under the bottom of the front of the condenser. The Zeiss have one chrome knob in the bottom front and another slider on one side- the slider has a little lock nut which is loosened before and tightened after centering.

Move the adjustments and watch the rings. If the proper rings are in place (assuming 40X objectives, Ph2 for Zeiss and #3 for Nikon), the two rings are the same size (occasionally one may be slightly thicker) and are perfectly concentric (or superimposed).

If the rings are not aligned, align them. Having the rings in perfect alignment is of paramount importance. Centering the rings is always left to last.

When the rings are centered, remove the telescope, replace the eyepiece, and the setup procedure is finished!

NOTE

On some condensers, when the rings are centered, the field iris may be slightly off center again. Do not reset the field iris: it may de-center the phase rings. The phase rings are always the most important! If one plans on buying a microscope, one might want to determine whether the two centering adjustments are interactive or independant (which is preferred)>

PROBLEMS AND SOLUTIONS

Fuzzy Image.....	Clean optics, adjust fine focus Usually greased ocular or smudged objective
Low Contrast.....	Center phase rings, clean optics Dirty eyepiece or objective
No Field Iris Visible....	Move condenser focus knob* Should nearly touch bottom of slide Open partially and inspect centering
Donut field iris.....	Move condenser focus knob*
Field iris not centered..	Find and use condenser-centering knobs
Field Iris Unevenly Lit..	Center light bulb
Phase Rings Fuzzy.....	Focus centering telescope
Graticule Fuzzy.....	Focus eyepiece or unscrew top lens
Graticule Dirty.....	Disassemble eyepiece & clean graticule
Graticule Not Straight...	Turn the eyepiece
Bright Ring Unevenly Lit.	Light bulb centering follow manufacturers instructions
Mottled Image.....	Dirt underneath glass above field iris. Such dirt is just above iris blades
No Light.....	Open field iris, place lens tissue over the glass and ensure light is coming to condenser
No Light.....	Make sure condenser iris is open. On some microscopes, a closed iris will block light, obscuring the annular diaphragm

Zeiss Note

A Zeiss microscope has a flip-up lens on the top of the condenser, used to alter the numerical aperture. The lens should be fully up when using the 40X objective. Two small tabs move the lens in and out and there is no detent position. Thus, a very slight bump of the tabs will cause an otherwise perfect image to deteriorate slightly or to fade to black, depending on the degree of bump. If all else fails, move the tabs and make sure the top lens is FULLY up!

Nikon Note

As mentioned earlier, after the phase rings are centered, the field iris may not be centered. Do not re-center the field iris. Live with it!

General Note

Routine Adjustment Procedure

for

Setting Up a Phase-Contrast Microscope

Photography and Photomicrography by

Catherine Dense-Goldberg

Stephen Bayer, Sr.

Microscope Graphics by

Dick Carlson

Objective

Using the material contained in this course module and after two hours practice using the microscope, the student shall demonstrate the ability to properly align a phase-contrast microscope to asbestos-counting status according to the requirements set forth in NIOSH Method #7400.

This presentation discusses the routine procedure for adjusting a phase-contrast microscope. Certain adjustments should be performed every time a new sample is to be analyzed.

TITLE GRAPHIC

Following this presentation, two hours of practice will be allocated for learning the adjustment procedure. The student will then be expected to demonstrate an ability to perform the adjustment procedure.

OBJECTIVE GRAPHIC

The three steps to be demonstrated are:

1) Focus on the sample

2) Adjust the field iris

3) Center the phase rings

GRAPHIC

In this program, you will see the microscopist perform these adjustments and view the effects through the microscope. Pay close attention to the location of the microscopist's hands.

MODEL AT SCOPE

Before looking into the microscope for the first time, raise the condenser until the top lens is nearly flush with the stage. The top condenser lens should nearly touch the bottom of the slide.

CU RAISING CONDENSER

Select an appropriate objective- 40X is typical for the method.

CU FINGERS SELECTING OBJ

Select the appropriate annular diaphragm. Number two is appropriate for Zeiss.

CU SELECTING #

Clip the sample of interest into the mechanical stage and use the mechanical stage controls to move the slide to an area suitable for viewing.

CLOSE UP MODEL HANDS

The first step is:

Focus on the sample.

GRAPHIC

Before looking into the microscope, look closely, from the side of the microscope, at the objective-to-slide distance. Using the focus knobs, move the slide until the cover glass nearly touches the objective.

Then, look into the microscope and focus.

When the 40X objective is in focus on the sample, the distance between the lens and cover glass is about 0.4 of a millimeter. Be very careful not to crush the objective into the slide.

CU OBJ-SLIDE DIST

Look into the microscope and, using the fine-focus knob, proceed to focus on the sample. Note the fine-focus control.

Remember the required turning direction for increasing the distance between the objective and cover glass. Initial focus should be achieved by increasing the distance while looking through the microscope.

MODEL LOOKING IN

Focus is now complete Do not refocus until the entire procedure is completed.

(3 SLIDES)
COMING INTO FOCUS

Although the image seen here may look fine to beginners, the image is actually quite low in contrast because the field iris and phase rings are not properly adjusted.

The second step is:

Adjust the field iris.

This step has several substeps: GRAPHIC

A) Close the field iris

B) Focus the field iris

C) Center the field iris

D) Open the field iris to the edge of the field of view.

Steps B and C are interchangeable.

The field iris is used to limit the illumination beam to the exact diameter as the entire image seen through the microscope, which is called the field of view.

Focussing the field iris establishes the condenser's point of focus at the specimen plane.

Reach down for the iris control. The iris is normally somewhat open. The iris will be closed to bring the image of the small opening inside the field of view.

A field iris consists of overlapping blades. Some have six and others eight. The field iris determines the diameter of the illumination beam. With six blades, the iris opening would appear hexagonal. With eight or more, the image is nearly circular.

(3 SLIDES)
MODEL CLOSING IRIS

Through the microscope, a fairly-distinct spot of light should be seen against the dark background. Notice that the sample is still in focus. One should be able to see the edges of the field iris blades. The strange looking image seen here is illuminated by an out-of-focus and off-center field iris.

CLOSED IRIS THRU SCOPE

By moving the condenser-focus knob, the edges of the iris blades can be made distinct.

CU COND FOCUS KNOB

Notice the edges of the iris blades.
Color fringing is often present and is normal.

FOCUSSED EDGES

The iris-centering knobs can now be used to approximately center the field iris.

CU IRIS CENT. KNOBS

In any practical sense, this iris image is centered and in an acceptable state of focus. Do not worry about being slightly out of focus. Simply make the image as crisp as possible.

FOCUSSED & CENTERED

The correct image appears as a bright spot of light against a black background. A donut, circle, or dark spot against a bright background means that the iris is out of focus.

The final adjustment for the field iris cannot be seen here, because it is outside the realm of the photomicrograph's image. The edge of the iris is opened to the edge of the field of view.

REGULAR CORRECT FIELD

If the centering needs fine adjustment, do so. The final goal of step #2 is that the iris has been focussed, centered, and opened equal to the diameter of the field of view.

This step places the condenser at the point of focus and blocks stray light which improves the image contrast.

The third, and last step is:

GRAPHIC

Center the Phase Rings

Centering the rings requires four steps:

A) Insert the centering telescope

B) Focus the telescope

C) Align the rings

D) Remove the telescope

Temporarily replace one of the eyepieces with the phase-ring centering telescope.

MODEL LEANING AWAY

The phase rings consist of a bright ring, found in the condenser, called an "annular diaphragm".

MACRO OF RINGS

The dark ring, called a "phase-shifting element", is inside the objective lens.

Although the rings are at two different locations, optically, the rings appear to be at the same location, one atop the other.

MODEL LOOKING IN

To see the rings sharply, two hands are used to focus the telescope on the rings.

The rings should appear as distinct as those seen here-- if the telescope is properly adjusted. There are at least <u>two</u> reasons the rings may not highly visible.

If the sample is very light, no light is scattered and the inside of the objective is dark. The dark ring or phase-shifting element is then hard to see.

If the observation area is very heavy, then the bright ring or annular diaphragm becomes vague and diffused.

RINGS THRU SCOPE

Under reasonable conditions, the sample scatters some light inside of the objective, the dark phase-shifting element ring is easy to see against the background, and the rings are clear and in focus.

Do not expect the rings to be focussed without adjusting the telescope. That takes takes two hands. Some lenses slide in a friction mount and others twist in a threaded mount.

CU MODEL'S HANDS

The focussed rings seen here are not centered. Centered means that the rings are perfectly aligned, exactly concentric, one atop the other.

For phase contrast to work as designed, the rings must be in perfect alignment. The bright ring or annular diaphragm must pass through the dark ring or phase-shifting element.

RINGS NOT CENTERED

Again, the rings seen here are not perfectly aligned.

This is the resultant image. There are many particles present and a few fibers also. They cannot be seen because the rings are not properly aligned.

WASHED-OUT IMAGE

Two centering adjustments can be found on nearly any microscope. Generally, on Nikons and Zeiss, the adjusters are near the outside portion of the condenser. The adjusters move only the bright annular diaphragm ring. By continuing the adjustment process, the two rings can be made concentric.

CU ADJUSTING KNOBS

Here are the same rings in a good state of alignment. Note that the bright annular diaphragm ring is a little thinner or narrower than the dark phase-shifting element ring. This condition varies between manufacturers. Others. make may have rings of nearly identical thickness. The important observation in this slide is that the two rings are concentric and that the light from the bright ring passes through the dark ring.

CENTERED RINGS

Finally, remove the telescope and replace the eyepiece or ocular

This completes the standard and routine steps for adjusting typical phase-contrast microscopes before counting an asbestos sample.

After about one day's practice, performing these three simple steps becomes easy and rapid.

CORRECT IMAGE

Usually, phase rings remain in alignment. Only a quick look through the telescope is required to find out, requiring about three to five seconds.

Condenser focus can vary from sample to sample because slide preps are not always the same thickness. The field iris centering will not change often. When experience gained, adjusting the field iris takes about five or six seconds.

In summary, three steps, for adjusting a phase contrast microscope, were described. The steps should be performed just before a sample is counted. When these steps are completed, one proceeds with the counting process.

MODEL LOOKING IN

The three routine steps for setting up a microscope for counting status are:

GRAPHIC

#1: Focus on the sample

#2: Adjust the field iris

#3: Center the phase rings

After the procedures are completed, one might wonder how image acceptability can be determined. Method 7400 specifies the use of a special image-quality test slide designated the HSE/NPL Mark II. This image is the test slide viewed with a 10 power objective.

HSE/NPL @ 100×

The 4 vertical lines intersect 2 pairs of horizontal lines. Elsewhere, outside the field of view, is another set of 4 vertical lines. The rectangle enclosed by these finder lines contains the test lines which are invisible at 100 power.

Here is the test slide using the 40X objective. Notice the coarse finder lines, much larger now. Also note the series of very fine parallel lines. These are termed "Set #1" even though they are not actually numbered on the slide.

HSE/NPL 400X #1

The first set may be seen on the left and the second set on the right. If the room lights are not low and the projection lens is dirty, the second set may not be visible. Method 7400 states that the first three sets of test lines must be completely visible. The fourth or fifth may only be partially visible.

HSE/NPL 400X #1&2

A microscope unable to render the fourth set partially visible is too low in resolution to use for fiber counting. A microscope capable of seeing, at least partially, the sixth set is too high in resolution. In either case, microscopes having either of these two image characteristics should not be used for Method 7400.

As a final note, phase-contrast optics are calibrated to work best using a certain color of light- usually green. Using the proper narrow-band filter not only improves the image contrast, but also reduces chromatic aberration in achromatic objectives. Consult manufacturer's data for the proper filter- the color recommended is nearly always green.

GREEN FIELD IMAGE

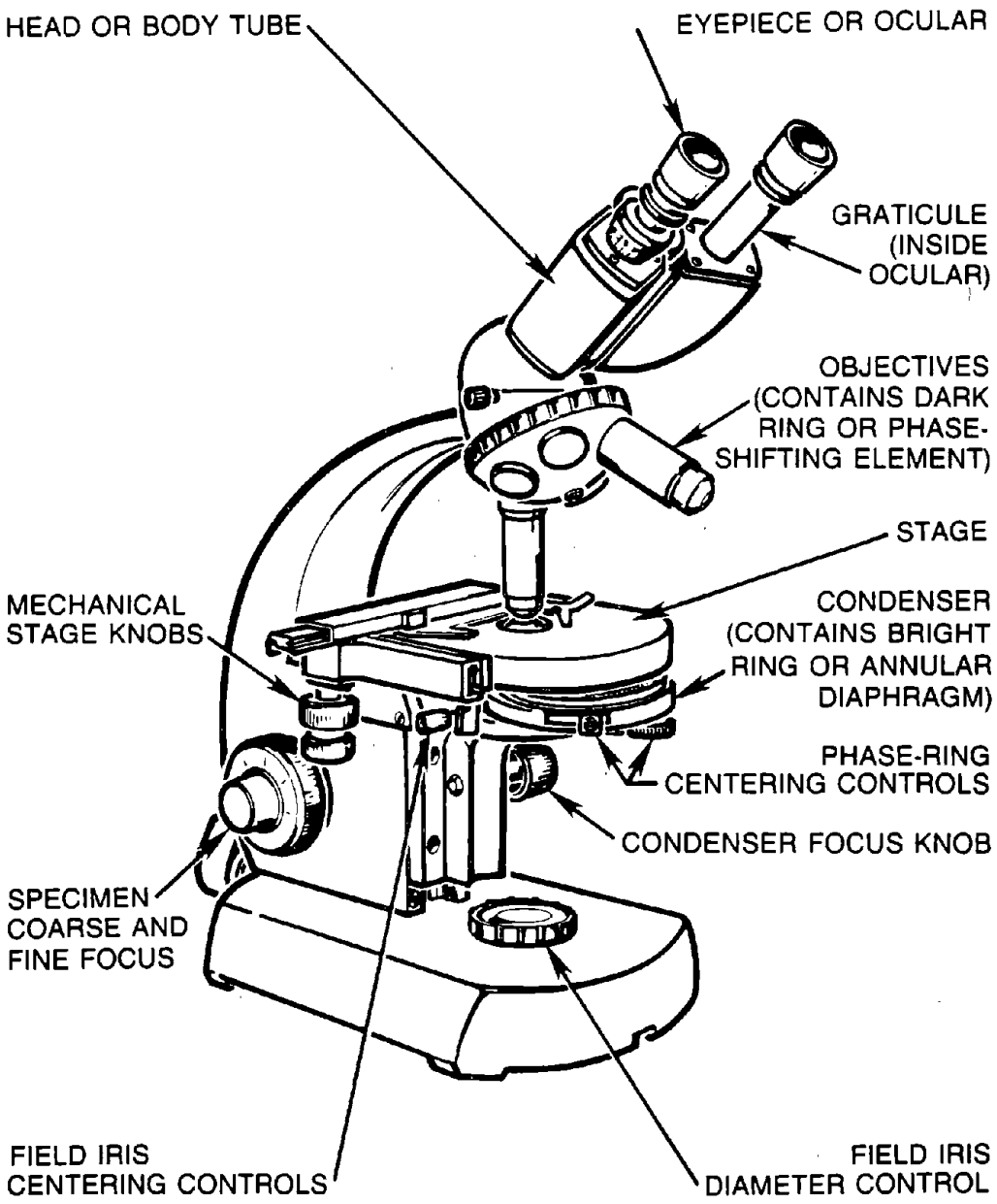
Proceed in adjusting your microscopes. Beginners should use the 10X objective and appropriate annular diaphragm. Later, when the process is better understood, change to the 40X objective and remember to select the proper annular diaphragm.

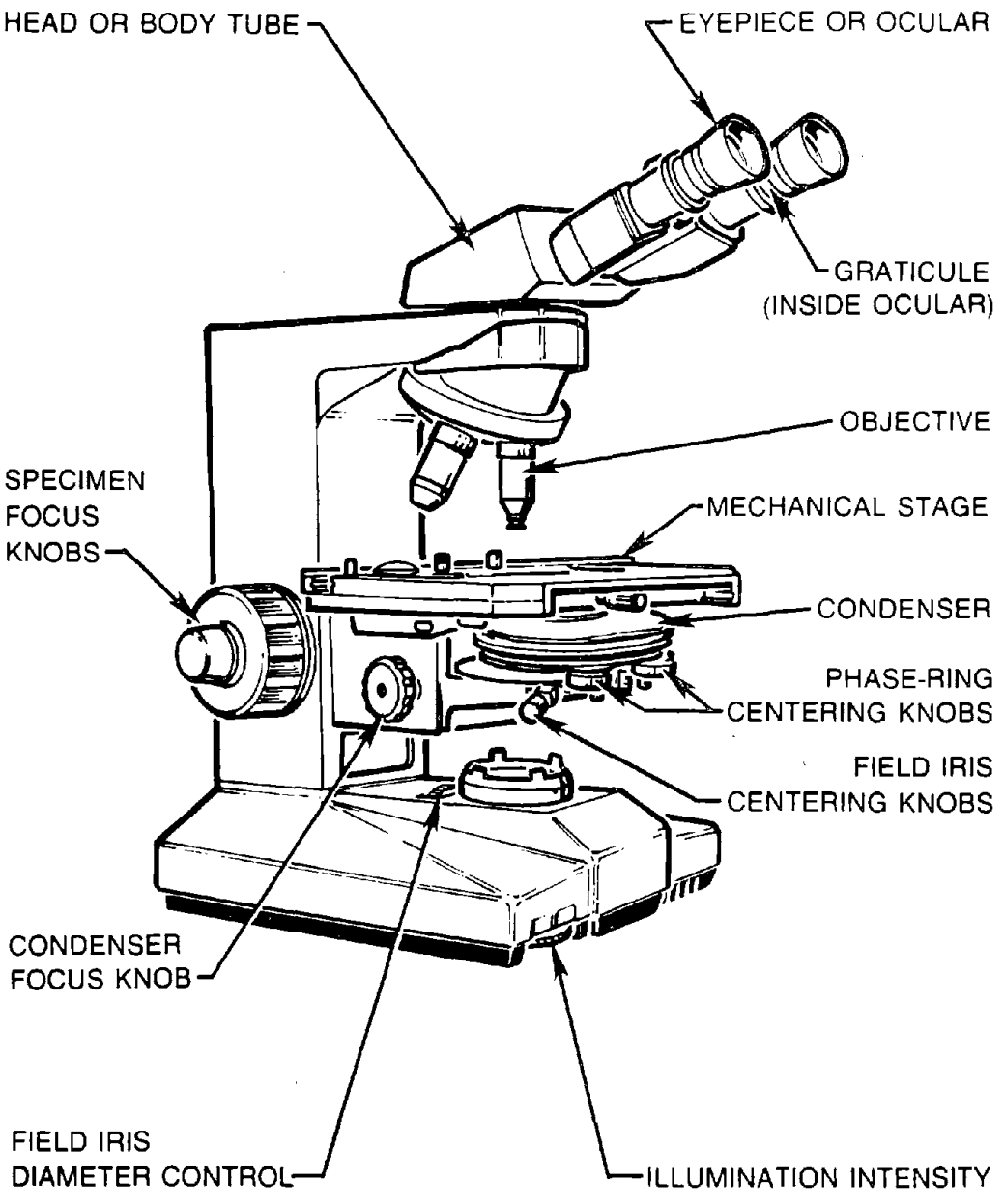
MODEL AT SCOPE

There is no need, in real life operations, to first set up at 100X and later repeat the process at 400X.

Carefully read the manufacturer's instructions for light bulb replacement, alignment, etc. This program is not meant to be a comprehensive guide for correcting all possible microscope maladies.

Manufacturer's literature and advise is very important.


HEAD OR BODY TUBE
EYEPIECE OR OCULAR
GRATICULE (INSIDE OCULAR)
OBJECTIVES (CONTAINS DARK RING OR PHASE-SHIFTING ELEMENT)
STAGE
MECHANICAL STAGE KNOBS
CONDENSER (CONTAINS BRIGHT RING OR ANNULAR DIAPHRAGM)
PHASE-RING CENTERING CONTROLS
CONDENSER FOCUS KNOB
SPECIMEN COARSE AND FINE FOCUS
FIELD IRIS CENTERING CONTROLS
FIELD IRIS DIAMETER CONTROL


HEAD OR BODY TUBE
EYEPIECE OR OCULAR
GRATICULE
(INSIDE OCULAR)
OBJECTIVE
SPECIMEN
FOCUS
KNOBS
MECHANICAL STAGE
CONDENSER
PHASE-RING
CENTERING KNOBS
FIELD IRIS
CENTERING KNOBS
CONDENSER
FOCUS KNOB
FIELD IRIS
DIAMETER CONTROL
ILLUMINATION INTENSITY

Graticule Calibrations

December 1987 Stephen G. Bayer

1000 um = 1 mm 0.001 mm = 1 um

If one custom orders* a Walton-Beckett graticule, three items are required:

1) the glass disc diameter (usually 19 or 21 mm)
   note: required to fit the eyepiece

2) version (G-22 for A counting rules)
   (G-24 for B rules)
   note- different aspect ratios!

3) Actual field diameter (in mm)
   note: this magnifies to 100 um diameter

The actual field diameter is the actual diameter of the circular counting field (on the graticule's glass disc) such that, when the graticule is installed in the eyepiece, the counting field's diameter becomes 100um (± 2 um). To determine the actual dimension, one needs a graticule with which to make initial dimensional determinations (a Porton, for example). The type of graticules is not important except that the image must have a measurable dimension (ruling out a cross-hair graticule, for example).

One makes three measurements. The Porton's length must be measured (in micrometers) inside the microscope which is adjusted to counting status. Then, one removes the Porton from the eyepiece and measures and records the same dimension in millimeters (also measure the diameter of the glass disc so it will fit inside the eyepiece). By simple proportion, the basic equation to calculate the size of circle needed is:

$$\frac{\text{Porton actual mm}}{\text{Porton magnified um}} = \frac{\text{Walton-Beckett actual mm}}{\text{Walton-Beckett magnified um}}$$

* To retrofit an older microscope, when purchasing different eyepieces, to have a graticule in a camera's optics, etc.

Suppose a Porton is 4.53 mm long on the glass and, through the microscope, the same dimension is magnified to 108 um (i.e. 0.108 mm). The equation becomes:

$$\frac{4.53 \text{ mm}}{108 \text{ um}} = \frac{\text{W-B actual dia. mm}}{100 \text{ um}}$$

By multiplying both sides by 100 um:

$$\frac{100 \text{ um} \times 4.53 \text{ mm}}{108 \text{ um}} = \text{Walton-Beckett diameter mm}$$

Compare this equation to the one on page 7400-12 item 7! L_a is actual dimension of the reference graticule, L_o is the magnified (optical) dimension of the reference graticule, and "D" is the desired magnified diameter of the Walton-Beckett (100 um)!

To see if the diameter is now correct, the graticule is installed in the microscope and measured through the microscope. Measure the diameter with a stage micrometer. The basic equation to determine the field area is Pi r^2! Since r is one half the diameter,

in millimeters:

$$3.1416 \times \left\{\frac{\text{Diameter}}{2}\right\}^2$$

in micrometers:

$$3.1416 \times \left\{\frac{\text{Diameter um}}{2} \times \frac{1 \text{ mm}}{1000 \text{ um}}\right\}^2$$

PROBLEMS

If the actual length of a Porton graticule is 4.5 mm ad, under magnification, the same dimension is magnified to 0.152 mm, what would be the ordering diameter of the Walton-Beckett's counting field?

Answer: _____ mm

The specified tolerance is 98 - 102 micrometers. Calculate the corresponding field areas:

For dia. = 98 um, the area is _____ mm^2

For dia. = 0.102 mm, the area is _____ mm^2

Porton Graticule Calibration

Probability is high that you will not have the need to calibrate or use a Porton graticule. However, since the vast majority of samples counted over the past twenty years were counted using a Porton, there is a chance you may be required, for example, to back-check previous information for accuracy. It may be handy to be familiar with the calibration procedure.

The Porton graticule has a mathematical design based around a slightly-ambiguous concept termed "a unit of length". By design, the Porton is 200 "units of length" long: by convention, this is usually expressed as 200L, where L symbolizes unit of length. From the length, any dimension on the graticule can be calibrated.

Given, the length in millimeters:

$$\text{Field area} = \left\{\frac{\text{length mm}}{2}\right\}^2$$

To determine the diameter (um) of a given circle:

First, find 1 L (one unit of length) in um:

$$1\ L = \frac{\text{length mm}}{200} \times \frac{1000\ \text{um}}{\text{mm}}$$

The diameter of circle # n = $1L\sqrt{2^n}$

In other words, take the length in millimeters, divide by 200, and then multiply by 1000 to find one unit of length in micrometers. Then, by multiplying the square root of two (raised to the power of the circle's number) by one unit of length (in um), one now has the diameter of the circle of interest.

Example: a Porton is 0.159 mm long:

$$\text{Field area} = (\ 0.159\ \text{mm} / 2\ )^2 = 0.00632\ \text{mm}^2$$

Find one unit of length in um:

$$1L\ \text{um} = \frac{0.159\ \text{mm}}{200} \times \frac{1000\ \text{um}}{1\ \text{mm}} = 0.795\ \text{um}$$

To find the diameter of the fifth circle:

$2^5 = 32$: the square root of 32 is 5.657.

Multiply L X $\sqrt{2^n}$:

0.795 um X 5.657 = 4.5 um

The diameter of circle #5 is nearly 4.5 micrometers.

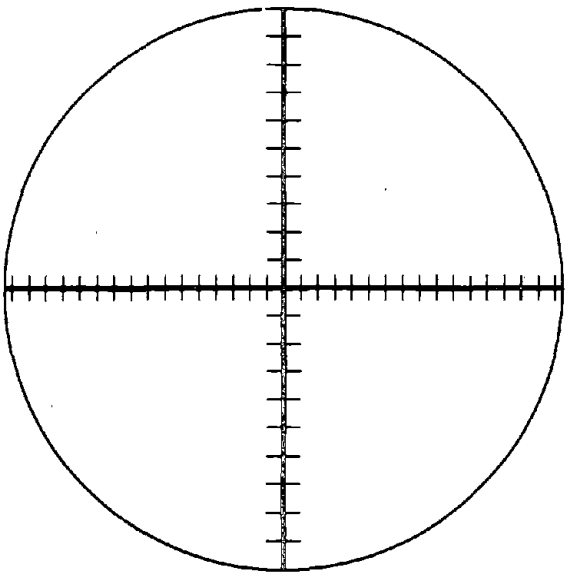
PROBLEM:

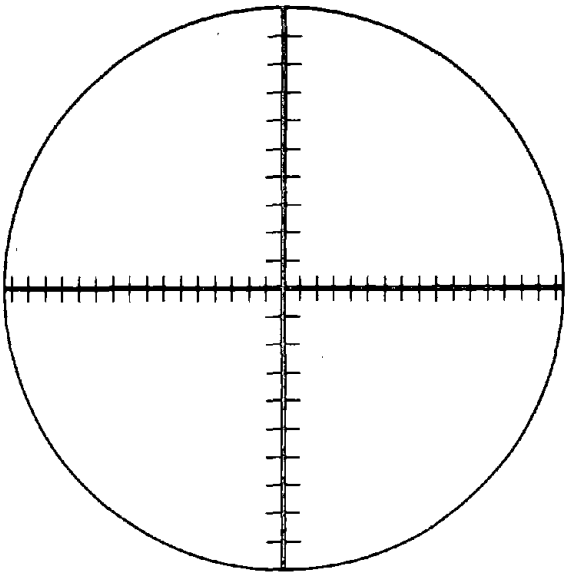
The magnified length of a Porton graticule is 0.149 mm. Determine:

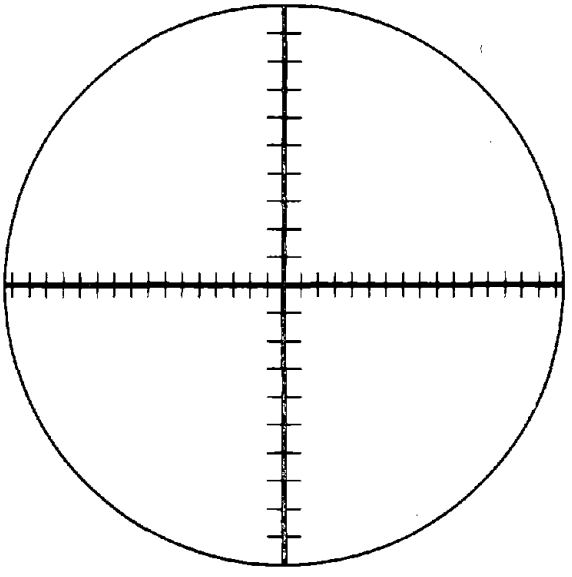
Field Area: _____ mm

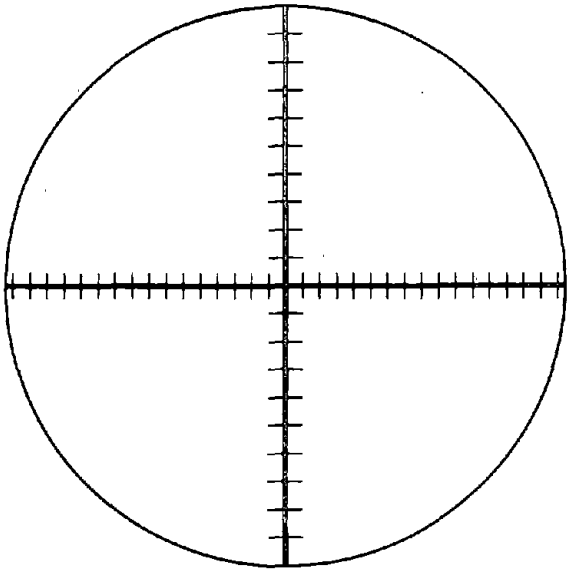
The circle closest to 5 um: Circle # _____

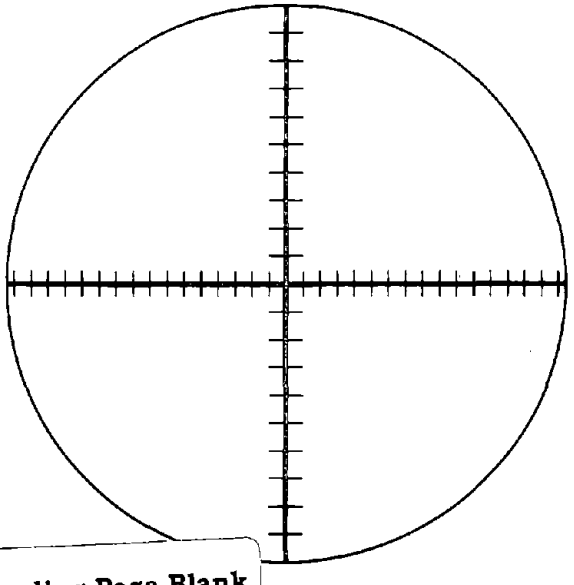
The field area of my microscope is _____ square millimeters

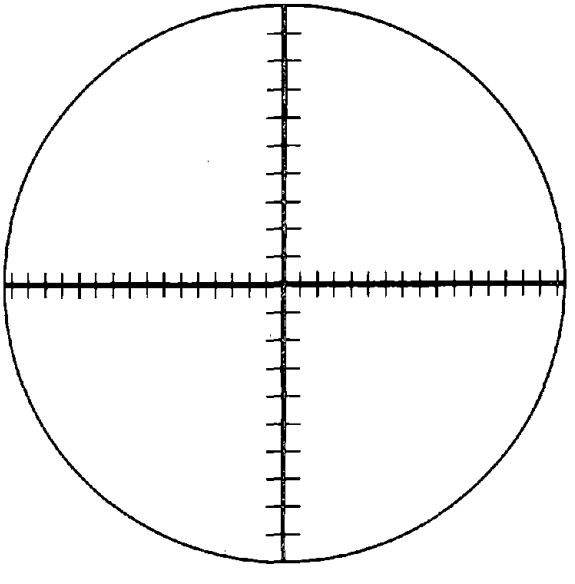












THE FILTER MOUNTING PROCEDURE

Stephen G. Bayer, Instructor

Objective: Describe the equipment, chemicals, and procedures used to create a slide preparation of asbestos which was collected on a cellulose ester filter. Given an assortment of slides, correctly identify the acceptable preparations.

This program describes the standard method used by NIOSH and OSHA for preparing mixed-cellulose-ester filters for fiber counting under phase-contrast microscopy. This method was developed in the 1960's as part of the industry-wide epidemiological survey of asbestos workers. It has an established performance record of dissolving the filter and fixing the sampled particulates in place without significantly affecting the sample characteristics or distribution. The advantages of the method are that the procedure is easy to learn and requires only 15 minutes for preparation time. The major disadvantage of the method is that the sample preparation is short lived. Preferably, the sample should be mounted and counted within the same day. Since, at most, only 1/6 of the filter is used when preparing a slide mount, the remainder of the filter should be permanently stored for future recounting if needed.

WARNING

According to the NIOSH Registry of Toxic Effects of Chemical Substances:

Dimethyl Phalate (Pthalic Acid, Dimethyl Ester) has exhibited teratogenic effects on rats and has an OSHA standard of 5 mg/m^3 in air.

Diethyl Oxalate (Oxalic Acid, Diethyl Ester) has no OSHA standard and no toxic criteria other than rat oral LD50: 400 mg/kg.

Skin contact should be avoided and adequate ventilation provided to comply with the DMP OSHA standard of 5 mg/m^3 8-hour time-weighted average.

Once the sample has been received, it must be mounted on a microscope slide for eventual viewing. The individual who mounts the filter controls a major step in the analytical process. If the slide preparation is carelessly prepared, it can destroy any useful results of the microscopic evaluation.

There are several myths in common circulation concerning slide mounting. Among them are these. The NIOSH mounting liquid makes the filter become invisible because the refractive indices of the liquid and the filter are the same. False, the solution dissolves the filter and hardens it into a plastic. The next myth is that the NIOSH method causes the sample to require excessive focussing up and down. False. It is advantageous to focus ±5 μm above and below the sample plane because fine features blink on and off attracting attention as they go in and out of focus. This requires about 1/4 turn of the fine focus knob and is not unreasonable considering the magnification is over 400X. Last is the misunderstanding that it doesn't matter how you mount the filter as long as it disappears. Perhaps the last myth is most important to clarify.

There are many ways to make a filter vanish such that the sample can be observed. The filter can be ashed, dissolved in a solvent,

dissolved in a solvent vapor, or immersed in a liquid of matching refractive index. All of these techniques allow the sample to be viewed without interference from the filter matrix. The key word, though, is "view". Viewing often means observing a sample to see what it looks like, determine the size distribution, and interpret optical phenomena for identification. Counting requires one more very important criterion that many other mounting techniques lack—sample security.

The asbestos concentration is determined by counting the number of fibers per unit area on the filter. It is very important that any mounting procedure used for asbestos samples slowly glues the fibers and particles in place. Avoid any procedure that can wash the sample away or permit the sample to progressively spread out as the filter dissolves since sample spreading reduces the measured concentration.

Another problem related to sample mounting should be considered bad practice. Suppose a sample is taken on a large filter and is expected to have a low fiber density—in other words—a low count. If the sample is dissolved and refiltered onto a much smaller area, the fibers per unit area increases which would make the original sample more countable. There are two problems with this procedure. First, the sample distribution from the filtered solvent may not be nearly as good as the original airborne distribution. Second, asbestos fibers are friable—in other words, they can be ground up and break apart fairly easily. Even worse, some have used ultrasonic agitators which break down the size of the fibers and converts large noncountable fibers into myriads of smaller ones which are now countable. Avoid this practice.

When mounting filters, simply keep in mind a basic philosophical attitude: any alteration to the distribution or morphology of the sample directly affects the measured concentration. Follow directions and the NIOSH procedure will be simple to perform and produce reliable results.

The first step is preparing the mounting solution or medium. It is solution of pthalic acid dimethyl ester (commonly known as dimethyl pthalate or DMP) and oxalic acid, diethyl ester (commonly known as diethyl oxalate or DEO). It's called a solution because, for each milliliter of solution, 0.045 grams of clean white plain cellulose ester filter material has been dissolved in it.

Adding the filter material increases the watery solution viscosity to that of maple syrup. The filter material is near the saturation point of the solvents and, when the filter is mounted, the filter slowly dissolves from the bottom. The viscosity of the solution holds the particulates in reasonable position, the solvents evaporate and the filter becomes a transparent, optically homogenous slide preparation.

Here is the necessary equipment to prepare the solution.

Phthalic Acid, Dimethyl Ester (DMP)
Oxalic Acid, Diethyl Ester (DEO)
Clean cellulose-ester filter material
25 ml graduated cylinder
Wheaton balsam bottle
Chemical balance accurate to 0.01 gram
Silicone stopcock grease

To make 25 ml of solution, proceed as follows:

1. Weigh out the required filter material on the balance. 25 X 0.045 = 1.13 grams.

2. Dispense 12.5 ml DEO into the Wheaton balsam bottle.

3. One by one, drop the filters directly into the DEO making up, each with the glass rod.

4. Try not to allow a build-up of semi-dissolved filter material on the side of the bottle.

5. Keep adding and stirring singular filters maintaining a slurry of dissolving filters until the DEO is nearly saturated.

6. When your patience expires, add 12.5 ml of DMP and stir the solution.

7. Add, one by one while stirring, the remaining filters.

8. Coat the lid of the bottle with a film of stopcock grease to inhibit solvent evaporation which promotes premature decay and crystallization of the solution.

9. Place the glass rod in the balsam bottle, cap it, and clean up spills with acetone.

10. The solution will be ready to use when all filter media is uniformly dissolved. This takes from a few hours to a day depending on how well the filters were chopped up as they were being added.

When the solution is ready to use, it will have a milky appearance, somewhat transluscent, but should be uniformly consistent. It may then be used to mount the sample.

Here is the additional equipment needed:

1 X 3 inch frosted end slides (or 25 mm X 75 mm)

1 X 1 inch #1½ cover glass (or 25 mm X 25 mm)

Scalpel with #10 curved blade

Fine-tipped tweezers

Fire-polished glass rod

Lint-free lens tissue

Pencil

Sample

The basic objective of mounting is to use just enough solution to clear the filter segment cut from the filter so that after the filter dissolves, it retains it's original dimensions. Proceed as follows:

1. Place two sheets of lens tissue on the table in front of you.

2. Carefully clean the scalpel, tweezers, and glass rod with lens tissue. Place the utensils on one of the pieces of lens tissue.

3. Clean a slide by exhaling on it and wiping off the moisture with lens tissue and rest it on the other sheet of lens tissue.

4. Carefully clean a coverslip in the same fashion. Don't cut yourself or break it—it's only 0.17 mm thick. Rest it with one edge on the non-frosted end of the slide.

5. Uncap the balsam bottle and withdraw the glass rod letting the first large drop fall back into the bottle.

6. Let the second drop fall onto the center of the slide.

7. Return the rod to the bottle and replace the lid so that dust doesn't settle out in your solution.

8. Take the other glass rod and spread the drop into a triangle 1/2" on each side.

9. Clean the glass rod with lens tissue.

10. Remove the filter cassette's band, and separate the top and center section from the base.

11. Starting at the center of the filter, see-saw the scalpel blade and cut out to the edge.

12. Rotate the cassette 1/6—1/8 turn and cut again out to the edge so as to cut a pie shaped piece from the filter.

13. Clean the scalpel.

14. Using the tweezers, grasp the filter segment by the perimeter and place the sample dust side up on the solution triangle.

15. With the tweezers, place the cover glass on the filter. DO NOT PRESS DOWN! DO NOT MOVE IT!

16. Be sure to legibly write the sample # on the slide.

AN ASBESTOS SAMPLE FILTER CLEARING PROCEDURE

Paul Andrew Baron, Ph.D. and Geoffrey C. Pickford, M. Eng. Sc., C.I.H.

ABSTRACT

An acetone clearing technique for cellulose ester or nitrate membrane filters is presented. This technique has the advantages of increased safety and more consistent clearing than ones previously recommended in other methods.

The technique involves the use of a heated metal block in which a small amount of acetone is rapidly vaporized and ducted to the filter. This prevents spattering of acetone liquid on the filter, reduces the amount of acetone required and gives even and rapid clearing of the filter. Construction and operational details of the block and associated heating apparatus are presented.

BIOGRAPHIES

Paul Baron received a BSc in Chemistry from the University of Illinois in 1965 and a PhD in physical chemistry from the University of California, Santa Barbara in 1970 and is presently responsible for a research program at the National Institute for Occupational Safety and Health in aerosol measurements related to industrial hygiene. The current emphasis of this program is sampling and analysis of airborne asbestos fibers. His previous efforts at NIOSH included development and evaluation of various instruments for both gases/vapors and aerosols. He is an adjunct assistant professor at the University of Cincinnati and a member of several professional societies.

Geoffrey Pickford is a Certified Industrial Hygienist working with a large Australian company as Chief Industrial Hygiene Engineer. After receiving a BE in mechanical engineering at the University of New South Wales, he completed a Master's degree in engineering in 1972 at the same university. While in the present position, he has handled a broad range of industrial hygiene topics and had a major responsibility in the measurement and control of asbestos dust from 1972-1983. He continues to be actively involved with the National Standards and the National Occupational Health and Safety Commission Working Groups responsible for producing vaiours gas/vapor and aerosol measurement methods.

AN ASBESTOS SAMPLE FILTER CLEARING PROCEDURE

Paul Andrew Baron, Ph.D. and Geoffrey C. Pickford,* M. Eng. Sc., C.I.H.

Introduction

The method for analyzing filter sample concentrations for asbestos fibers involves chemically collapsing the cellulose ester or nitrate filter to provide a clear background against which to count the individual fibers with an optical microscope. The currently recommended method for accomplishing the filter clearing both in the United States (NIOSH Method 7400)[1] and in a number of other countries[2] is to use a stream of acetone vapor to clear the filter. This involves using a flask of boiling acetone to provide sufficient vapor to rapidly collapse the filter structure without distorting the dust deposit on the filter. This procedure has been criticized as being a potential fire hazard, especially in situations outside the laboratory. Another acetone clearing technique has been recommended by the U.K. Health and Safety Executive that consists of a boiling acetone reservoir with a condensing coil to prevent acetone escape.[3] This technique is safety but still uses a reservoir of hot acetone and requires the use of circulating water for cooling. A technique for clearing filters will be presented that allows rapid clearing with the use of a minimum amount of acetone liquid or vapor.

The basis for the technique is the rapid vaporization of a small amount of injected acetone liquid, approximately 0.20 mL, in a heated metal block. The vapor is then transported through the metal block and condensed on the filter. The condensation is rapid (the entire injection and clearing process takes less than 5 seconds) and occurs over the entire surface of the filter so that clearing is gentle and complete without shrinkage of the filter surface.

Equipment and Procedures

Figure 1 shows the overall layout of the apparatus currently in use. An SCR voltage controller (Cole Parmer R-2603-00) provides power to two 2" x 5" 50 watt insulated heating pads (Cole-Parmer R-3125-20). The heating pads and a surface thermometer (Fisher 15-170-10A) are attached with silicone glue to a machined aluminum block. The cross section of the aluminum block is shown in Figures 2 and 3. The 1/4-20 screw plugs the end of the connecting hole between the vaporization chamber and the condensation chamber to prevent vapor escape. An insulating teflon plug with a small inlet allows the insertion of the tip of an adjustable micropipette (such as the Eppendorf Digital). Acetone is injected into the vaporization chamber directly below the teflon plug. The vapor is ducted over to the filter where it condenses on the cooler filter and slide surfaces. The ducting has no direct path for liquid droplets to reach the filter while allowing the vapor to expand and diffuse so that the filter is exposed to a uniform plume of acetone. A slot in the bottom of the block allows the analyst to push a slide with the filter on it directly under the condensation chamber. The heights of the slot is sufficient to allow filters or filter segments that are not completely flat to enter without

*James Hardie Industries Limited, 65 York Street, Sydney, N.S.W. 2000, Australia.

touching the top of the slot. Recessed areas in the base of the block reduce heat loss to the surface on which the block is resting.

The voltage controller is turned on to begin operation and set to maximum for about five minutes to reach the operating temperatures of 70°C +/- 5°C. Care should be taken not to allow the block to overheat during this period since the block will then require additional time to cool off. At this time, the controller can be set at the final voltage setting. This setting should be determined for each operational system and location, but is approximately half the maximum voltage. When the block has reached operating temperature, filter clearing can begin. The block is placed on a smooth, clean, chemically unreactive surface (e.g., a glass plate) so that the slide holding the filter segment can be pushed in and out of the slot at the bottom of the block with ease. A slide with a filter or filter segment placed at the appropriate location is positioned at the entrance to the slot. A blank slide can be marked to act as a template showing the correct location of the filter segment.

When clearing one-fourth of a 25 mm filter, the pipette is loaded with 0.2 mL acetone and positioned at the hole in the teflon plug. The slide is pushed into the slot and the acetone is then immediately injected into the vaporization chamber. Note that if the uncleared filter is exposed to the heat longer than several seconds, it will start to curl up and the cleared filter may be distorted. The pipette is kept in the hole in the teflon plug to prevent acetone vapor from being released through the injection hole. The slide is left in the slot for approximately 3 to 5 seconds to allow complete clearing of the filter and then removed.

Discussion

The design details of the apparatus seem to be open to modification, as long as the basic considerations of safety and even exposure of the filter to acetone vapor are observed. The use of a temperature controller and thermistor sensor has been used to give better temperature control and eliminate the need to watch the device during warm-up. However, since the temperature of the aluminum block does not seem to be particularly critical, the added expense of temperature control may not be warranted. The apparatus has been operated at temperatures between 65 to 75°C with good results. At higher temperatures, the acetone boils more violently (and may even decompose) upon injection and liquid droplets can impact on the surface of the filter. At lower temperatures, the acetone tends to stay on the filter and slide for a longer time and the surface tension of the liquid draws dissolved filter material and particles away from the edge of the filter.

The amount of acetone needed for complete and consistent clearing varies with the size of filter segment being cleared. An injection of 0.15 to 0.30 mL works well for one quarter of a 25 mm filter. For larger or smaller segments, the amount should be increased or decreased and tested on blank filter segments.

It was found that one batch of filters produced cleared filters that were somewhat mottled in appearance. For this reason, it is suggested that filters from each batch be tested before sampling for asbestos.

Experience with hundreds of filters cleared with this technique has produced samples that were consistently flat, unwrinkled, fully cleared, and without air bubbles. This was not the case using the previous method, with which problems in these areas often necessitated preparation of additional slides.

After clearing the filter, final preparation of the sample involves covering the filter with triacetin and a cover slip, then waiting overnight to allow maximum clearing of the filter surface. An additional technique to speed up the preparation of acetone cleared asbestos filter samples has recently been described.[2] This technique involves heating the acetone fixed, triacetin covered filter for 15 minutes at 50°C to speed the clearing process. If only a few slides are being prepared, it is possible to place the triacetin covered sample slide on the aluminum block to obtain the increased clearing speed. The combination of the flash vaporization clearing technique and the one described here will allow safer and more rapid sample preparation of asbestos filter samples.

Precautions

Note that in spite of the reduction of acetone vapor release into the environment with this technique, the presence of liquid acetone is still a fire hazard and should be treated with a great deal of respect. If this technique is performed outside of a laboratory, it should be carried out in a well ventilated area in the absence of flames and spark sources. The volume of acetone liquid in current use should be kept to a minimum (less than 20 mL) and the liquid kept covered when not in immediate use.

Several considerations need to be observed if other heating systems are used. The voltage or temperature controller should be solid state to eliminate the high temperature and sparking of contacts in non-solid state controllers. The heating element should be well insulated electrically in order to prevent shorting to the metal block and be in good contact with the block surface to prevent overheating of the element. The wires should also be well insulated and secure to reduce electrical shock hazard to the operator. In areas where only 240 V line power is available, a reduced voltage control system is recommended.

Recommendations

The use of flash vaporization to limit the amount of acetone consumed during filter clearing provides a significant safety advantage. In addition to the increased safety, the wider and more homogeneous plume of acetone vapor impinging on the filter clears the filter in a more complete

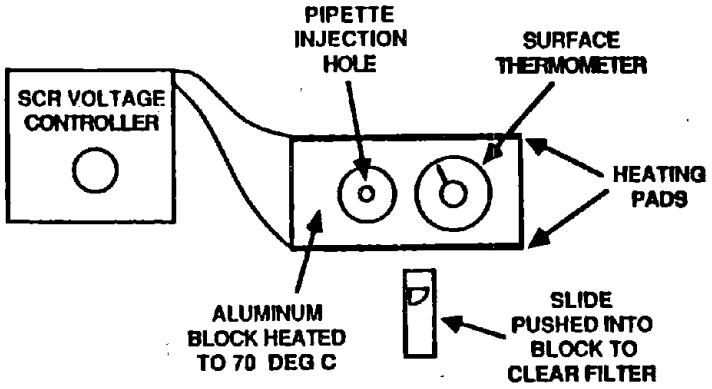
and consistent fashion than methods previously recommended. It recommended that this technique be included in a future revision of NIOSH Method 7400 and in other methods involving cellulosic membrane filter collapse for sample preparation.

REFERENCES

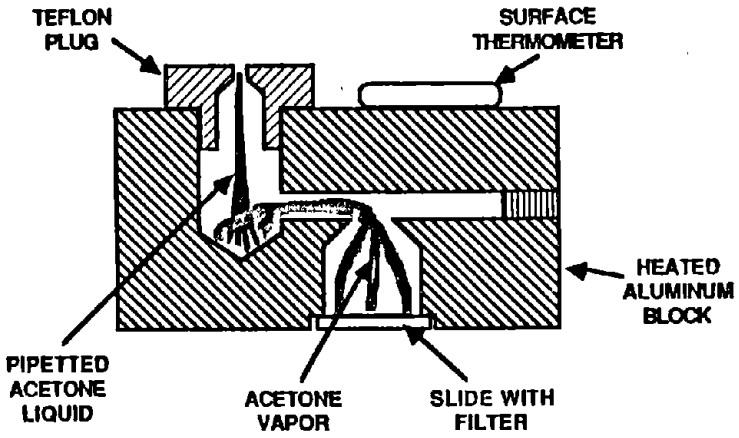
1. NIOSH: Fibers, Method 7400 (Revision 1). NIOSH Manual of Analytical Methods. 3rd Edition. NIOSH, Cincinnati, OH (1985).

2. Asbestos International Association: Reference method for the determination of airborne asbestos fibre concentrations at workplaces by light microscopy. AIA Health and Safety Publication, Recommended Technical Method No. 1. (1979).

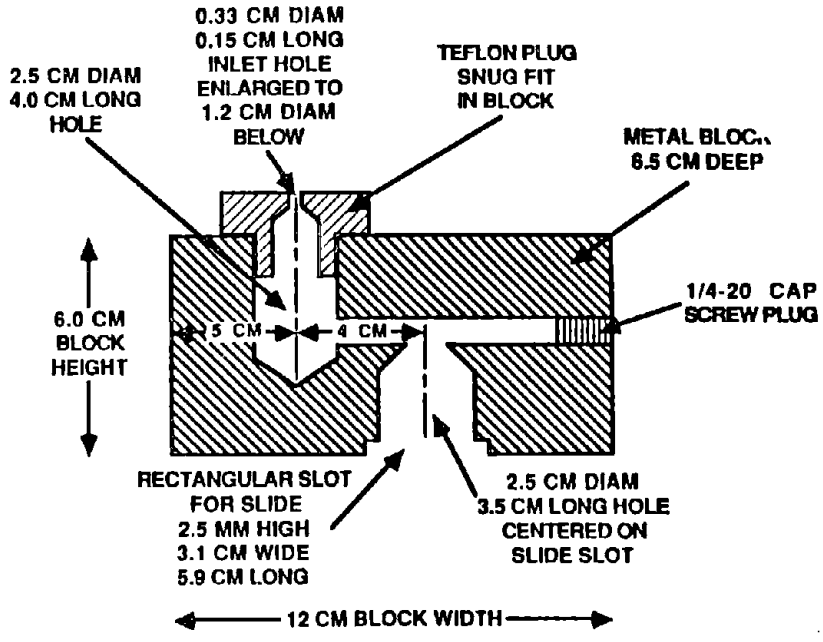
3. Health and Safety Executive: Method for the Determination of Hazardous Substances 39, Asbestos Fibres in Air, HSE, Occupational Medicine and Hygiene Laboratory, London (1984).



1. Diagram of filter clearing system.



2. Cross section of heating block during operation.



3. Dimensions of heating block.

QUALITY-CONTROL TESTING OF ASBESTOS COUNTS

BY STEPHEN G. BAYER

JUNE 1986

THIRD DRAFT

DISCLAIMER

The following information is a training resource used to illustrate concepts and stimulate thinking, discussion, and comment. This module represents the author's personal opinions and viewpoints and has not undergone formal NIOSH review. Please do not, in any official capacity, quote from nor reference this manuscript. Instead, complete the assigned reading and quote from appropriate sections in the official literature.

ASSIGNED READING: METHOD 7400

PAGE 7400-5, ITEMS 14 AND 15

PUB #79-127 (FIRST SECTION IN MANUAL)

PAGE 4 - 5

PAGE 47 - 48 SECTION 10.2

LECTURE PURPOSE:

Describe the theory and application of an equation used to determine whether or not two counts of a specific asbestos sample are statistically acceptable.

PERFORMANCE OBJECTIVES:

Given two measurements and a CV, perform a test for bias.

Determine whether the sample passes or fails.

Describe the characteristic shape of a graph illustrating the frequency distribution of the absolute difference between a series of paired asbestos counts.

INTRODUCTION

Given in publication 70-127 is the statement "It is very desirable for a counter to conduct a 'blind recount' for about 1 in every 10 filter wedges (slides) counted."

Method 7400, item 14b states "Perform blind recounts by the same counter on 10% of filters counted (slides relabeled by a person other than the counter)."

Although both of these statements refer to single counters performing a comparison, on pages 4 and 5 of publication #79-127, please note that the statistical test to be described relates also to comparisons between two separate individuals.

There are three different ways counts are compared. The first, and most common, is an individual performing a personal recount against an actual sample he or she had counted previously. Comparisons of this type should be made on 10% of all samples evaluated. The second quality check involves two or more individuals cross-comparing on an actual sample. The third approach involves all counters comparing on laboratory reference slides to determine the "in-house CV." The reference slides should be permanent slides of actual samples spanning a range of fiber densities and encompassing a variety of interference levels.

In the first two comparisons, the following equation is used to test counts (usually expressed in fibers/mm^2) for possible bias:

TEST VALUE = 2.77 X AVERAGE X CV

Using the average of two counts, if the difference between two counts numerically exceeds the test value, one of the counts is biased and the sample should be discarded. The remaining samples in the batch should also be recounted and tested in the same fashion. All samples failing the test should be discarded (interpret "discard the sample" as discard the analytical data associated with the sample).

PROCEDURE

Suppose a sample had been counted by two different people or counted by the same person twice. The average of the two data, the absolute difference between the two numbers, and a value for the coefficient of variation (CV) must be determined.

The first example will be based on a CV = 0.40. Counter A measured 225 fibers/mm^2 and counter B determined 575 fibers/mm^2. The average of the counts is 400. The difference between the two counts is 350. The count-testing equation calculates the test value of 443.

TEST VALUE = 2.77 X 400 X 0.4 = 443

Since the difference between the counts is 350 and the test value is 443, the counts pass the test.

Failing the comparative test means that one of the measurements may be biased (or, in other words, suspect of being incorrect).

Note also that the CV of 0.40 in the examples is a practical worse-case value. One should use CV's determined from repetitive counting of reference slides.

The second example will revise the CV to 0.20. The following conclusion is drawn.

2.77 X 400 X 0.20 = 222

THE SLIDE FAILS THE TEST: Thus, the two counts are statistically acceptable with an associated CV of 40% - but not with a CV of 20%. As states earlier, the CV used is determined from historical in-house data.

STATISTICAL PERSPECTIVE

Following are actual counts, generated by students in the 582 course, from one of the reference slides.

SLIDE #1: AVERAGE: 430 FIBERS/MM^2 COUNTED 47 TIMES

LOWEST COUNT: 153 HIGHEST COUNT: 825 CV: 0.32

FIBERS/MM^2	FREQUENCY	PLOT
0 - 49		
50 - 99		
100 - 149		
150 - 199	1	0
200 - 249	1	0
250 - 299	6	000000
300 - 349	6	000000
350 - 399	7	0000000
400 - 449	9	00X000000
450 - 499	2	00
500 - 549	7	0000000
550 - 599	5	00000
600 - 649		
650 - 699	1	0
700 - 749		
750 - 799		
800 - 849	2	00
850 - 899		

Since the mean, the median (24th value indicated with an X), and the mode are all in the 400 - 450 group, the fiber counts are normally distributed. One could use conventional statistical equations to predict a range in which counts would fall--given various probabilities.

For example, if one were to perform the following calculation,

MEAN $\pm$ 2.77 X MEAN X CV

one would obtain two values which, with 99.5% confidence, would predict a range of future counts--NOT A DIFFERENCE BETWEEN COUNTS! Although this equation looks similar to the count-testing equation, it is predicting a RANGE BETWEEN two counts as applied to a normal, bell-shaped distribution of counts on a given sample.

The count-testing equation is somewhat similar in philosophy to the one used in compliance-testing. One of the compliance-testing equations calculates a SINGLE VALUE that, with 95% confidence, is not going to be exceeded. The compliance-testing equation also deals with the bell-shaped distribution of concentrations (or counts) associated with the analysis of a single sample.

For the BELL-SHAPED distribution, the 95% upper confidence value uses the equation:

MEAN + 1.645 X CV X MEAN

The count (concentration, etc.) calculated from this equation should not, with 95% confidence, be exceeded; however, there is a 5% probability that it may!

THEORY

The count-testing equation does not deal with the frequency distribution of counts. Rather, the statistic deals with the frequency distribution of ABSOLUTE DIFFERENCES BETWEEN COUNTS! As seen on the graphs included on the last three pages, the frequency distribution of absolute differences between counts is HALF OF A BELL SHAPE which changes the previous equation value from 1.645 to 2.77.

For slide #1, the greatest count difference that exists is between the highest count and the lowest count - 153 and 825 fibers/mm^2 respectively. One could say that one of the counts is only 19% of the other or that one count is 540% higher than the other. In either case, the difference seems staggering! Note that 672 is the absolute difference between the counts.

For n data, there are $n^2 - n$ possible combinations of two counts, each of which results from comparing a count to every other count except itself! Slide #1 was counted 47 times. There are 2162 possible combinations from which absolute differences can be determined--a task best left to the computer.

For convenience, one may catagorize the data by groups--differences between 0 to 49, 50 to 99, etc. While the frequency distribution of the differences is being assembled, it is easy to keep a running grand total as each group frequency is determined and then go on to calculate the current percent contribution toward the total number of possible combinations (termed cumulative %).

SLIDE #1: DISTRIBUTION OF ABSOLUTE DIFFERENCES BETWEEN COUNT PAIRS

2162 POSSIBLE COMBINATIONS

			FREQUENCY	CUMULATIVE %	
0	-	49	442	20.44	
50	-	99	432	40.42	
100	-	149	362	57.16	
150	-	199	312	71.6	
200	-	249	202	80.94	
250	-	299	166	88.62	
300	-	349	82	92.41	
350	-	399	58	95.09	ACTUAL 95% POINT
400	-	449	36	96.76	
450	-	499	30	98.14	
500	-	549	22	99.16	
550	-	599	14	99.81	
600	-	649			
650	-	699	4	100	

The test value, using the true mean and CV for slide #1, is:

2.77 X 430 X 0.32 = 381

Thus, purely based on the equation, mean, and CV, the test value predicts that there is a 95% probability that no two counts should differ more than 381. Looking at the actual frequencies, notice that 95.09% of the possible combinations of count differences is accumulated in the 350 - 400 group.

The test equation indicates, with a mean of 430 and a CV of 0.32, that there is only a 5% chance that two counts should differ more than 381. If the difference between two counts exceeds the test value, one (or perhaps both) of those counts biased.

This would be the case of the two extreme values discussed earlier which resulted in a difference of 672. Had these two individuals compared counts, the counts would have failed the test.

Is discarding the data justified simply because the two counts failed the test? Perhaps the difference results from problems with the counter(s) and not with the sample. One certainly has to wonder--is it worth it to go back and review the sample and see if the sample is work "salvaging" or, if in fact, the analyses should be "discarded" as prescribed by method #7400? Guidelines are not currently available because of the number of variables which can cause a failure of the test. Examples include interferences, poor sample deposition, careless counting, unusual fiber configurations, combinations of these, and more.

The fact that a QC failure has occurred should motivate an investigation as to WHY. Method #7400 requires that the remaining samples in the batch be recounted and likewise tested. All samples from the batch which fail the test should be discarded. This possibility should generate concern when count comparisons frequently exceed 70% of the test value. Granted, the counts pass--but the range between counts is significantly wide.

SUMMARY

2.77 X AVERAGE X CV

The test for bias in paired counts is associated with a 95% confidence limit as applied to a distribution of absolute differences between paired observations. The distribution has a characteristic shape appearing as HALF OF THE BELL SHAPE associated with normal distributions.

The test calculates a number--representing a count difference--that should only stand a 5% chance of being exceeded. If the actual difference between two counts exceeds the calculated test value, one (or both) of the counts is biased.

Slide #3 is left for classroom discussion.

PROBLEMS

1) COUNT A: 749 COUNT B: 296 CV 0.30 PASS OR FAIL?

2) COUNT A: 214 COUNT B: 63 AT WHAT CV WOULD THIS FAIL?

SLIDE #3 AVERAGE: 413 N = 54

LOWEST COUNT: 153 HIGHEST COUNT: 825 CV: 0.29

MEDIAN 400 MODE 400 - 450 GROUP

FIBERS/MM^2	COUNT FREQUENCY	ABSOLUTE DIFFERENCE FREQUENCY
0 - 49		652
50 - 99		658
100 - 149		490
150 - 199	1	412
200 - 249	1	268
250 - 299	7	178
300 - 349	9	82
350 - 399	9	48
400 - 449	11	24
450 - 499	2	24
500 - 549	7	14
550 - 599	5	10
600 - 649		
650 - 699	1	2
700 - 749		
750 - 799		
800 - 849	1	

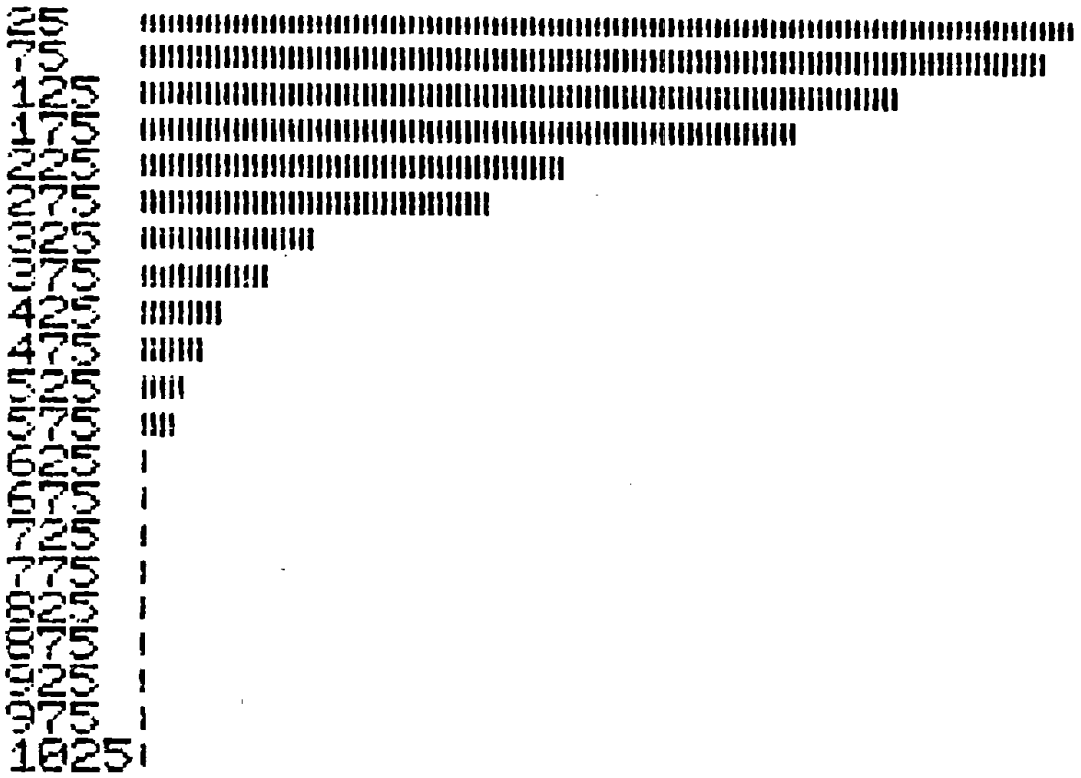
2862 POSS. COMBINATIONS

TEST VALUE: 332 ACT. 95% POINT: 300 - 350 GROUP

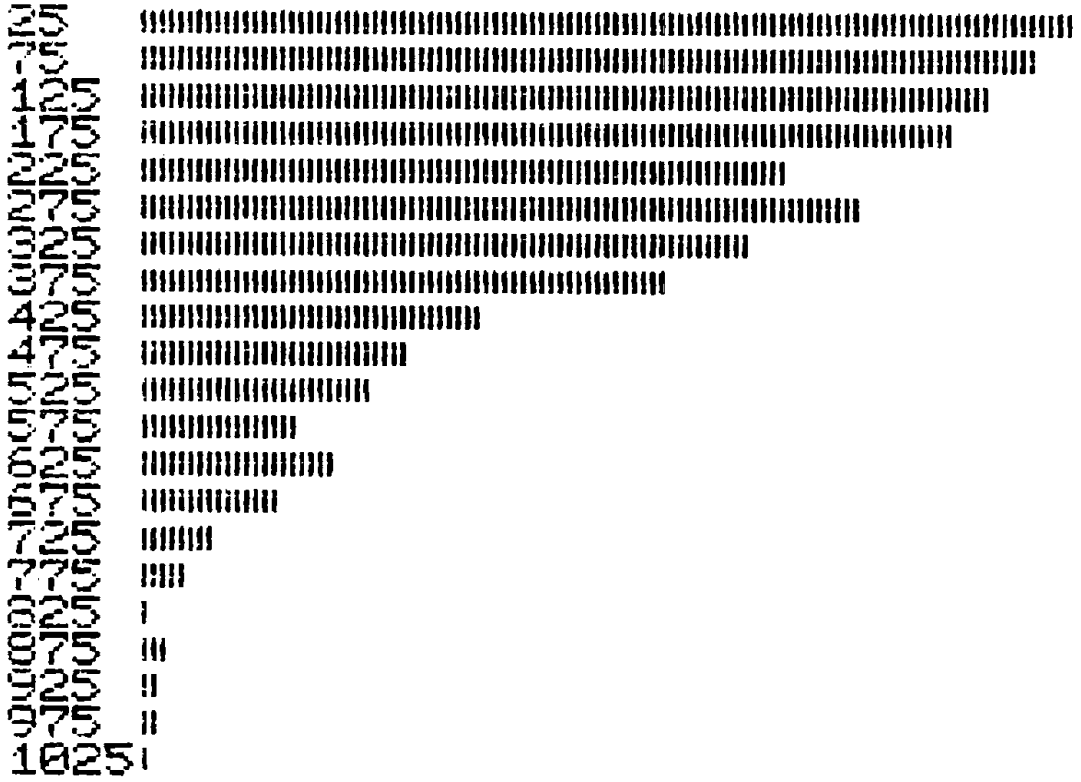
Attached are graphic displays of the frequency distribution of absolute differences of thirteen reference slides--including slides one and three. The group ranges were revised to centerline values--i.e., 25 for 0-49, 75 for 50-99, etc. The actual frequencies of the peaks of each bar in the graphs is not really important. The frequencies range from about 30 for some slides to over 800 on others. The peak heights of the 0 to 50 group were expanded and compressed to similar scale. The remaining group frequencies fall into scale by proportion. Note, for the most part, that the curves are half-bell shaped and very similar in appearance. The data for the statistical test are:

SLIDE #	MEAN	CV
1	430	0.32
2	734	0.31
3	413	0.29
4	193	0.73
5	513	0.51
6	248	0.46
7	340	0.44
8	436	0.30
9	436	0.25
10	416	0.50
11	308	0.34
12	373	0.48
13	399	0.25

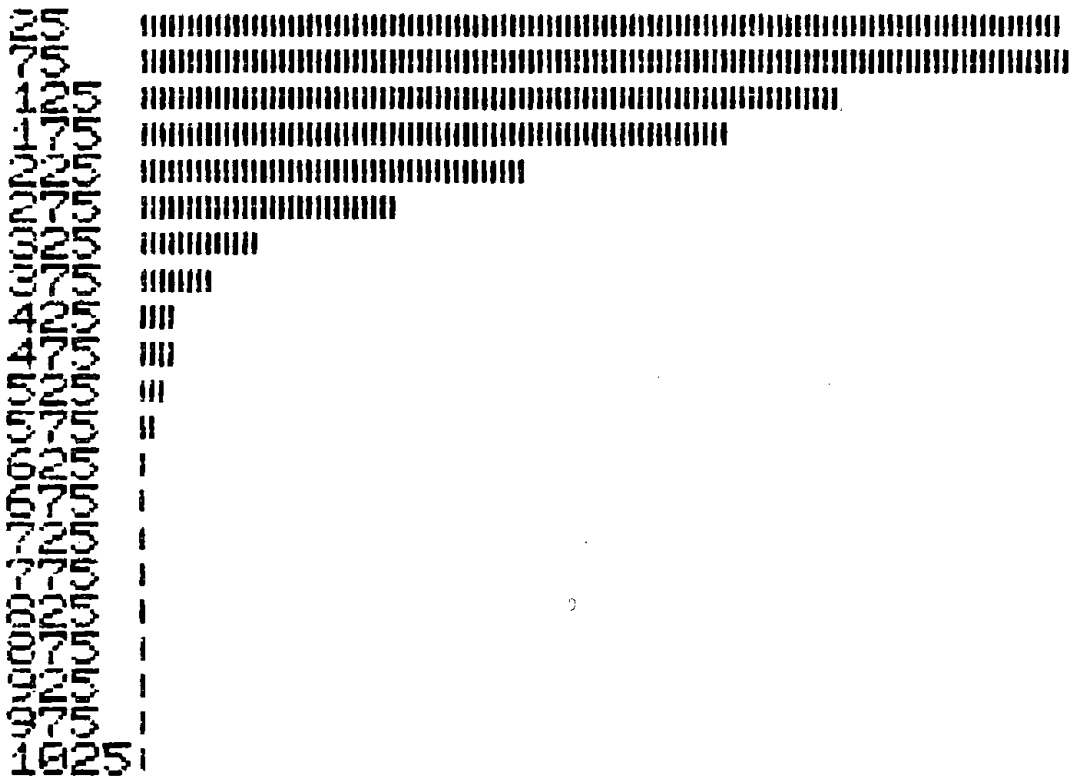
Perhaps you might like to calculate a few of the test values and observe where the count difference would fall on the actual curves.



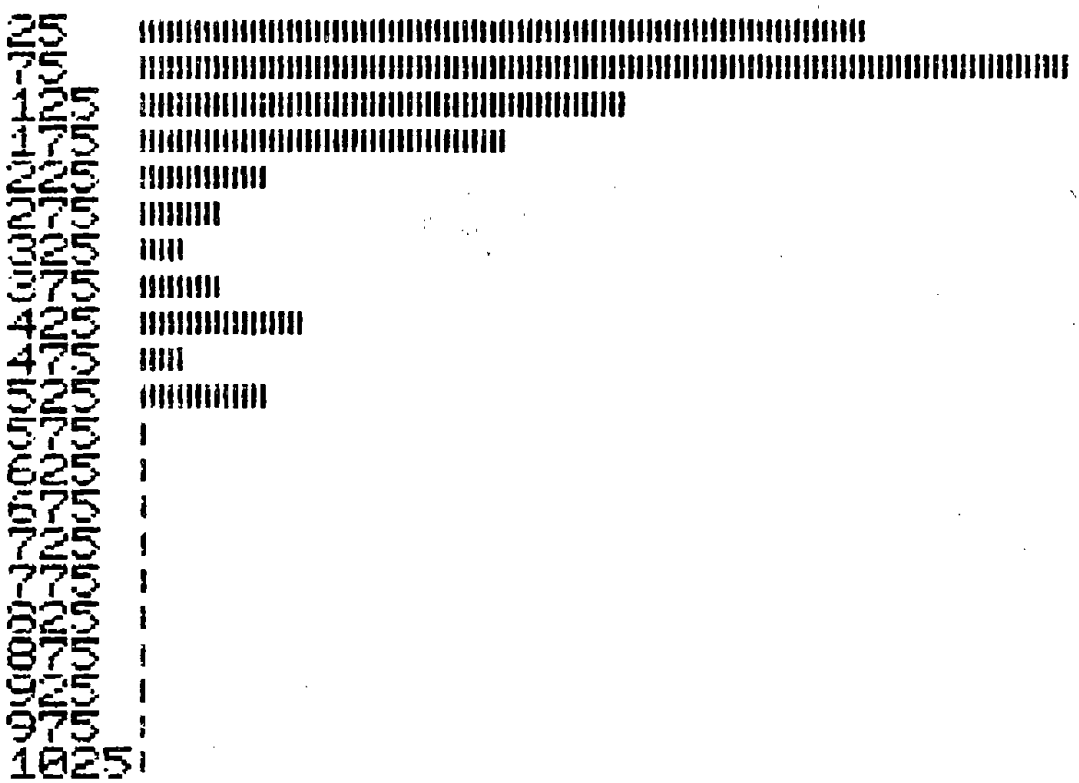
SLIDE #1



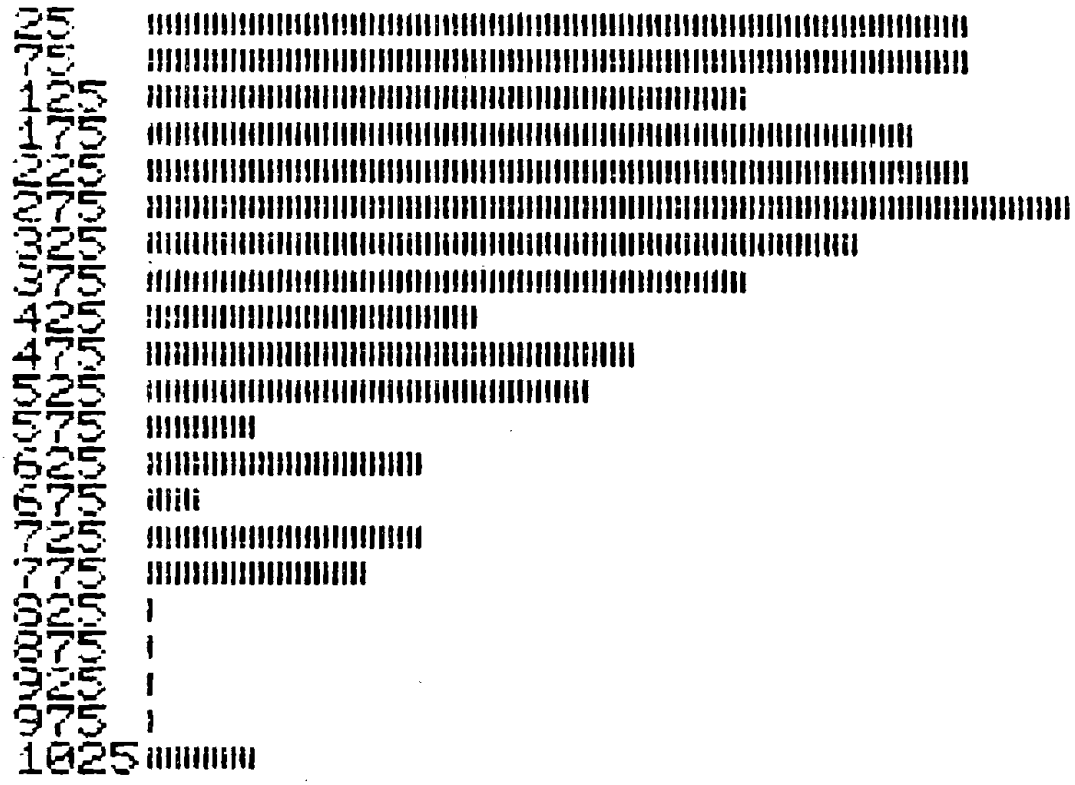
SLIDE #2



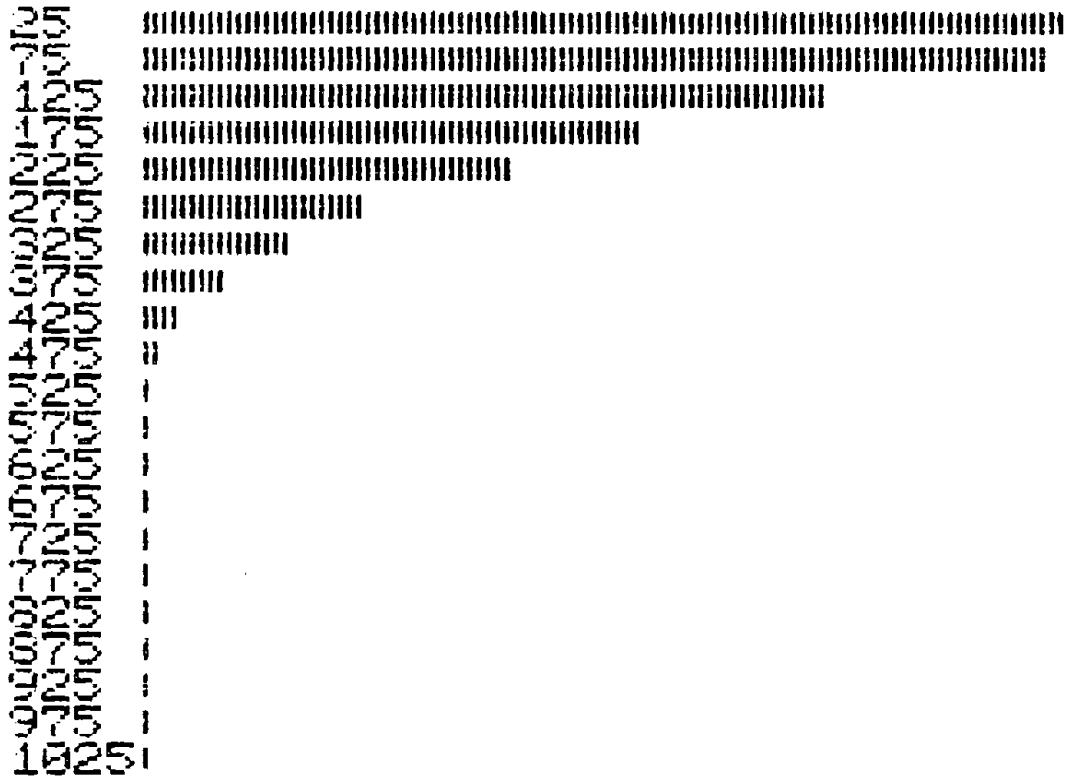
SLIDE #3



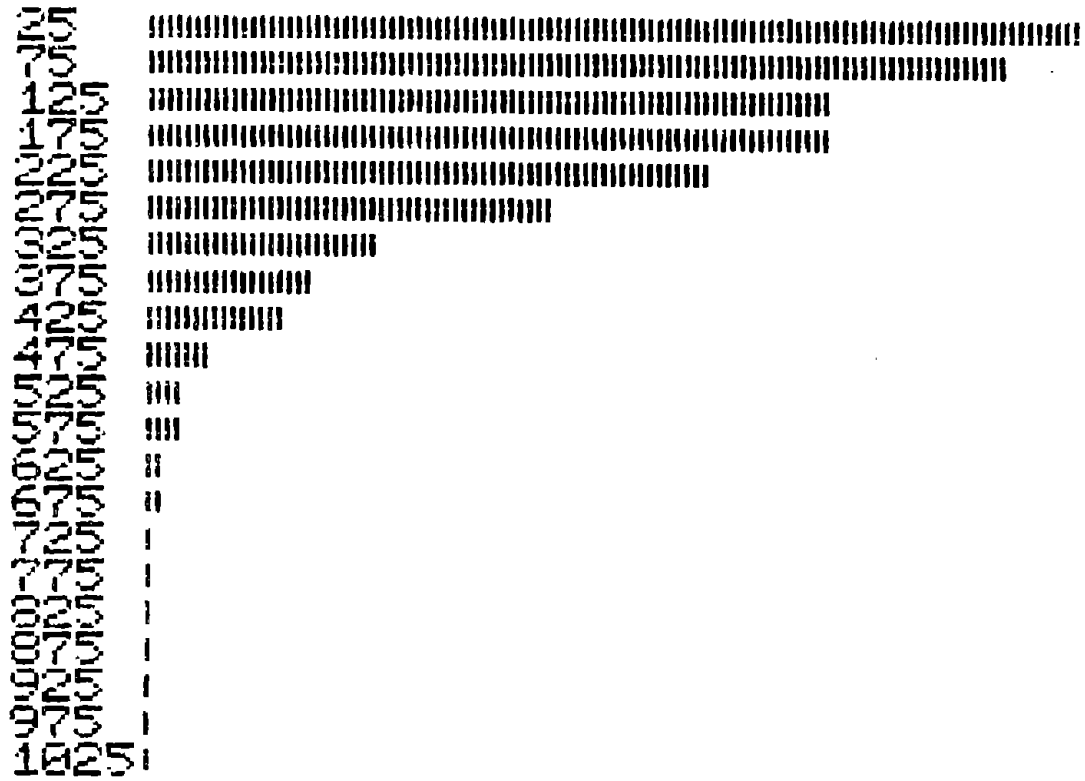
SLIDE #4



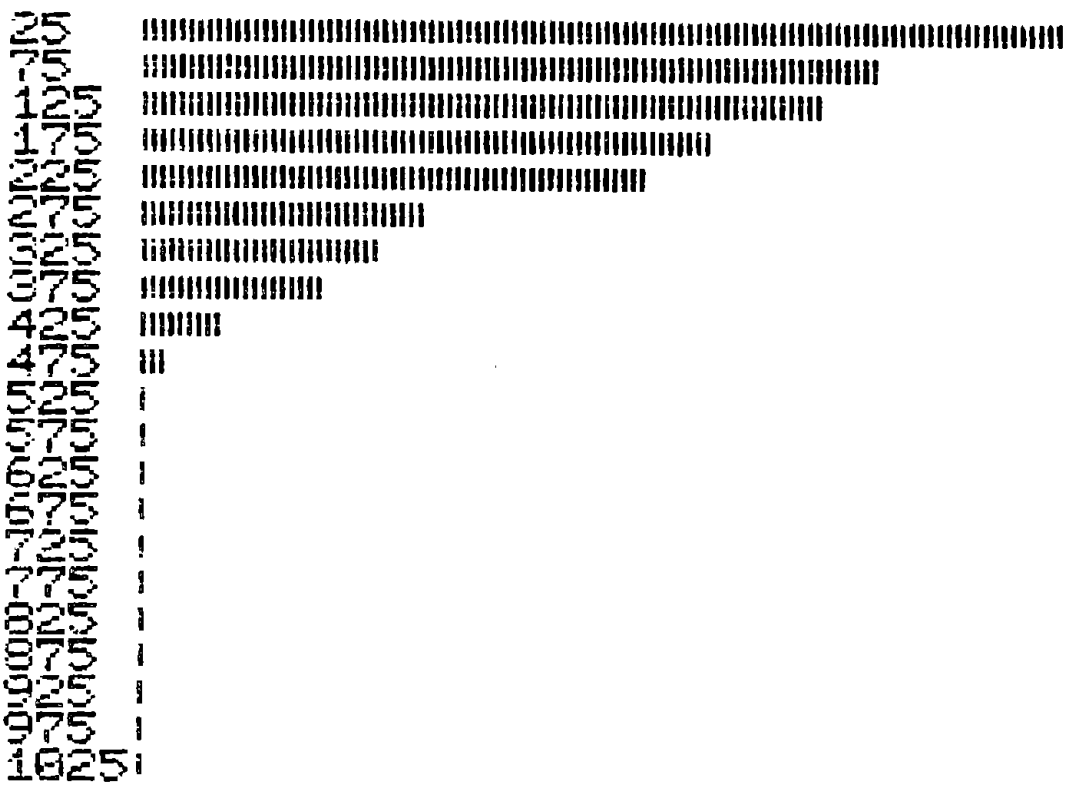
SLIDE #5



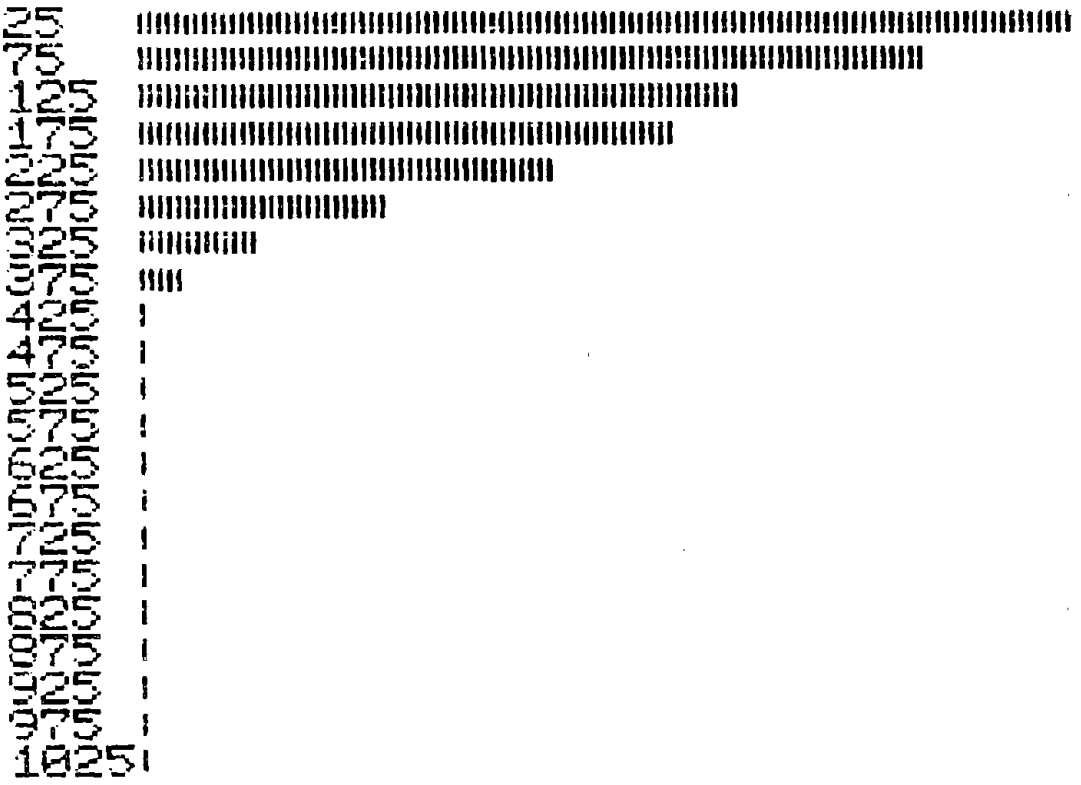
SLIDE #6



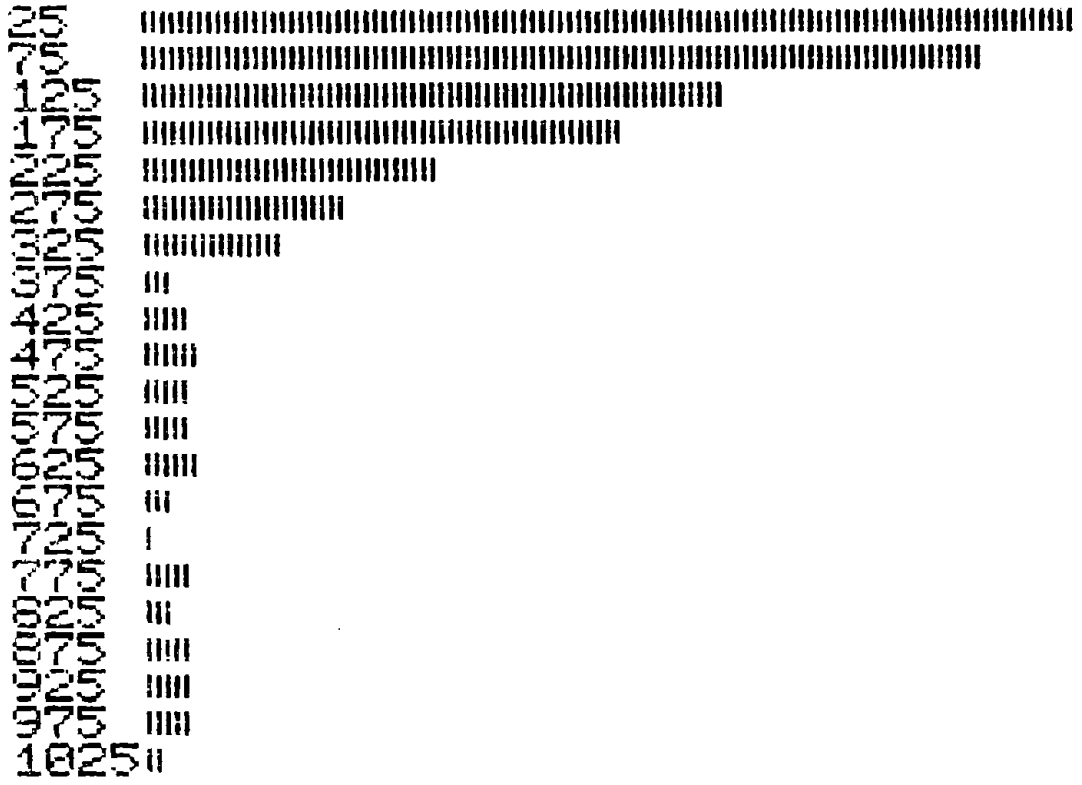
SLIDE #7



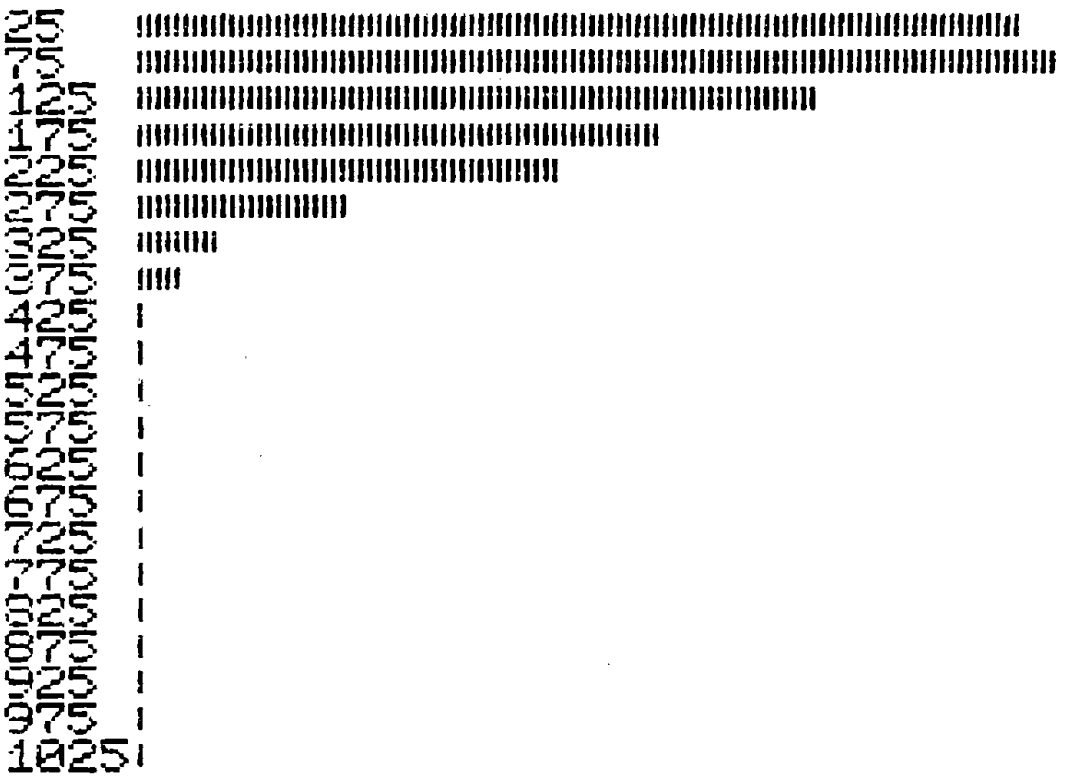
SLIDE #8



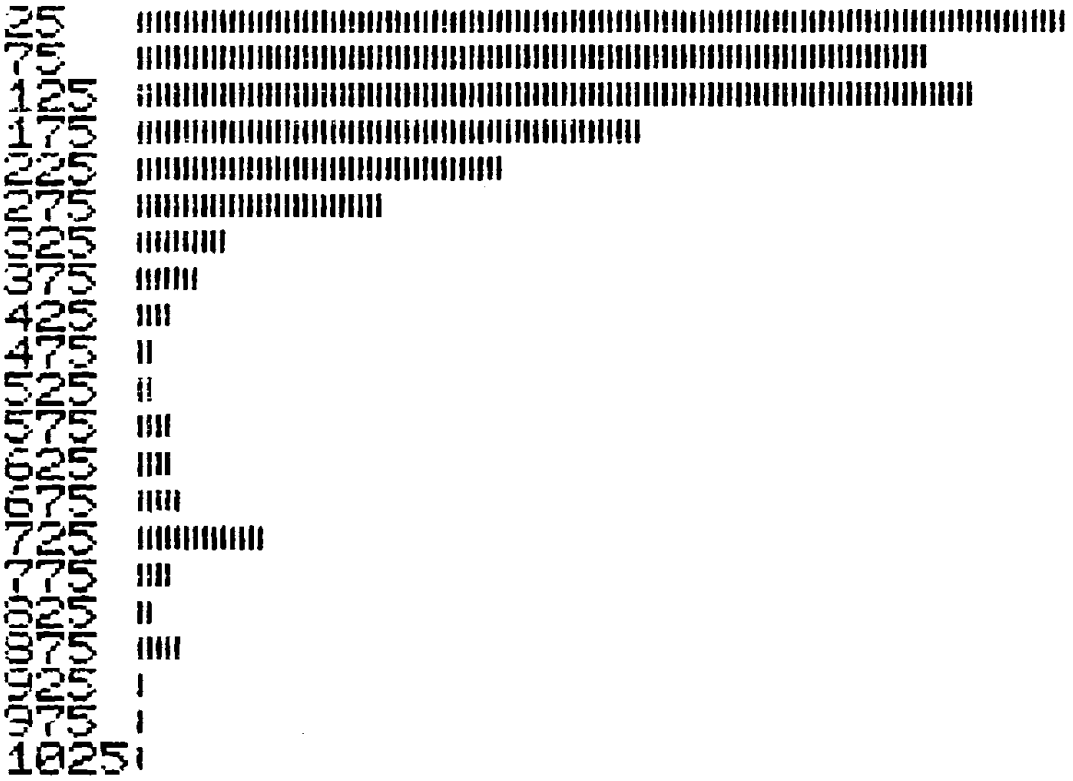
SLIDE #9



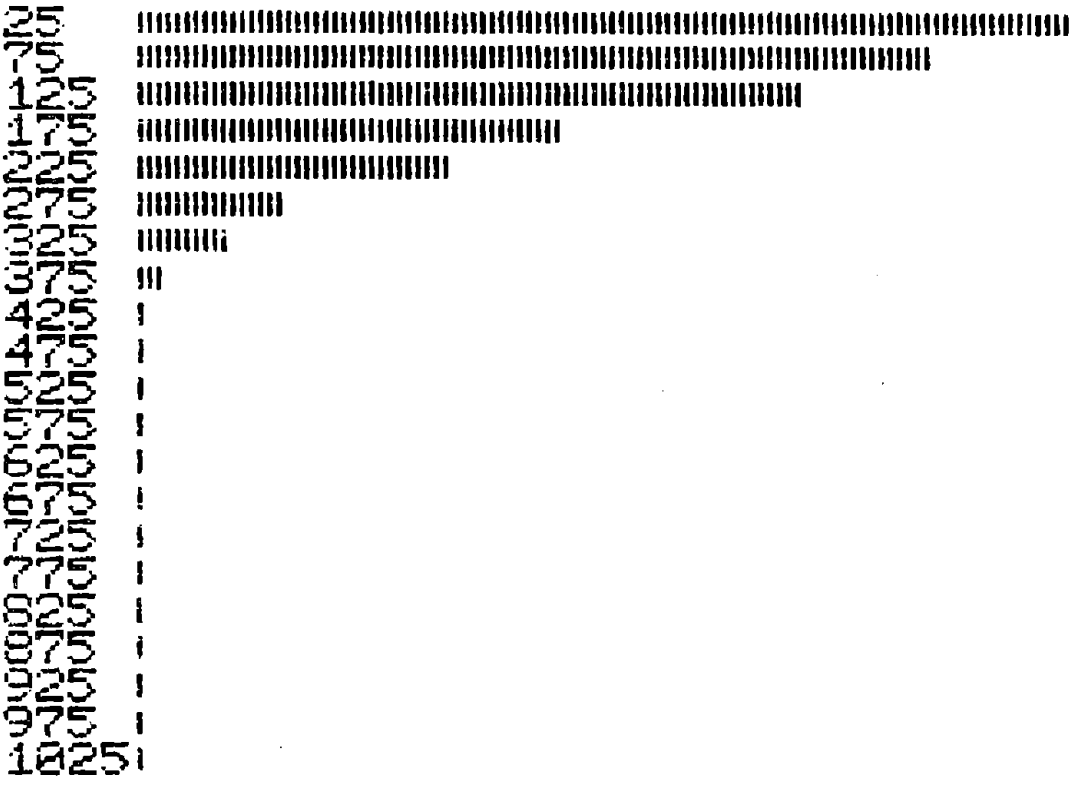
SLIDE #10



SLIDE #11



SLIDE #12



SLIDE #13

FORMULA: various

M.W.: various

ASBESTOS FIBERS

METHOD: 7402
ISSUED: 8/15/87

OSHA: 0.2 asbestos fibers (>5 μm long/mL) [1]
NIOSH: 0.1 asbestos f/mL [2]
ACGIH: 0.2 crocidolite; 0.5 amosite; 2 chrysotile and other asbestos, f/mL

PROPERTIES: solid, fibrous

SYNONYMS: actinolite asbestos [CAS #13768-00-8], grunerite asbestos (amosite) [CAS #12172-73-5], anthophyllite asbestos [CAS #17068-78-9], chrysotile asbestos [CAS #12001-29-5], crocidolite asbestos [CAS #12001-28-4], tremolite asbestos [CAS #14567-73-8].

SAMPLING

SAMPLER: FILTER
(0.8-to 1.2-μm cellulose ester membrane, 25-mm diameter; conductive cassette)

FLOW RATE*: 0.5 to 16 L/min (step 4)

VOL-MIN*: 400 L @ 0.1 fiber/mL (step 4)
-MAX*: (step 4)
*Adjust for 100 to 1300 fibers/mm² (step 4)

SHIPMENT: routine (securely packed to reduce shock)

SAMPLE STABILITY: stable

FIELD BLANKS: 10% (≥2) of samples

ACCURACY

RANGE STUDIED: 80 to 100 fibers counted

BIAS: not determined

OVERALL PRECISION (s_r): see EVALUATION OF METHOD

MEASUREMENT

TECHNIQUE: MICROSCOPY, TRANSMISSION ELECTRON (TEM)

ANALYTE: asbestos fibers

SAMPLE PREPARATION: modified Jaffe wick

EQUIPMENT: transmission electron microscope; energy dispersive X-ray system (EDS) analyzer

CALIBRATION: qualitative electron diffraction; calibration of TEM magnification and EDS system

RANGE: 100 to 1300 fibers/mm² filter area [3]

ESTIMATED LOD: 1 confirmed asbestos fiber above 95% of expected mean blank value

PRECISION: 0.28 when 65% of fibers are asbestos; when adjusted fiber count is applied to PCM count, s_r for combined method is 0.20 [14].

APPLICABILITY: The working range is 0.04 to 0.5 fiber/mL for a 1-m³ air sample. The method measures asbestos fibers of the smallest diameter (<0.05 μm) but allows comparison of fiber counts to be made with phase contrast light microscopic (PCM) data when fiber diameters are rigidly defined.

INTERFERENCES: Non-asbestiform amphiboles may interfere in the TEM analysis if the individual particles have aspect ratios greater than 3:1. These interferences can only be eliminated by quantitative zone axis electron diffraction analysis. High concentrations of background duct interfere with fiber identification.

OTHER METHODS: NIOSH Methods 7400 (dated 8/15/87) (phase-contrast microscopy) and 7403 (polarized light microscopy) are designed for use with this method.

REAGENTS:

1. Acetone. See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: field monitor, 25-mm, three-piece cassette with ca. 50-mm electrically-conductive extension cowl, cellulose ester membrane filter, 0.8- to 1.2-µm pore size, and backup pad.
   NOTE 1: Analyze representative filters for fiber background before use. Discard the filter lot if mean count is >5 fibers/100 fields. These are defined as laboratory blanks.
   NOTE 2: Use an electrically-conductive extension cowl to reduce electrostatic effects on fiber sampling and during sample shipment. Ground the cowl when possible during sampling.
2. Personal sampling pump, ≥0.5 L/min (see step 4 for flow rate), with flexible connecting tubing.
3. Microscope, transmission electron, operated at 100 kV, with electron diffraction and energy-dispersive X-ray capabilities, and having a fluorescent screen with inscribed or overlaid calibrated scale (Step 15).
   NOTE: The scale is most efficient if it consists of a series of lines inscribed on the screen or partial circles every 2 cm distant from the center.
4. Diffraction grating replica with known number of lines/mm.
5. Slides, glass, pre-cleaned, 25- x 75-mm.
6. Knife, #10 surgical steel, curved-blade.
7. Tweezers.
8. Grids, 200-mesh TEM copper, carbon-coated.
9. Petri dishes, 15-mm depth. The top and bottom of the petri dish must fit snugly together. To assure a tight fit, grind the top and bottom pieces together with an abrasive such as carborundum to produce a ground-glass contact surface.
10. Foam, clean polyurethane, spongy, 12-mm thick.
11. Low-temperature oxygen plasma asher.
12. Filters, Whatman No. 1 qualitative paper or equivalent, or lens paper.
13. Vacuum evaporator.
14. Cork borer, No. 5 (8-mm).
15. Pen, waterproof, marking.
16. Reinforcement, page, gummed.
17. Asbestos standard bulk materials for reference.
18. Carbon rods, sharpened to 1 mm x 8 mm.
19. Microscope, light, phase contrast (PCM), with Walton-Beckett graticule (see method 7400).
20. Grounding wire, 22-gauge, multi-strand.

SPECIAL PRECAUTIONS: Acetone is extremely flammable (flash point = 0 °F). Take precautions not to ignite it. Heating of >1 mL acetone must be done in a fume hood using a flameless, spark-free heat source.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line [5].
2. For personal sampling, fasten sampler to worker's lapel near worker's mouth. Remove the top cover from end of the cowl extension (open face) and orient sampler face down. Wrap joint between extender and monitor body with shrink tape to prevent air leaks. Where possible, especially at low %RH, attach sampler to electrical ground to reduce electrostatic effects during sampling.

3. Submit at least two field blanks (or 10% of the total samples, whichever is greater) for each set of samples. Remove top covers from the field blank cassettes and store top covers and cassettes in a clean area (e.g., closed bag or box) during sampling. Replace top covers when sampling is completed.
4. Sample at 0.5 L/min or greater [6]. Adjust sampling rate, Q (L/min), and time, t (min), to produce fiber density, E, of 100 to 1300 fibers/mm^2 [3.85•10^4 to 5•10^5 fibers per 25-mm filter with effective collection area (A_c= 385 mm^2)] for optimum accuracy. Do not exceed ca. 0.5 mg total dust loading on the filter. These variables are related to the action level (one-half the current standard), L (fibers/mL), of the fibrous aerosol being sampled by:

$$t = \frac{A_c \cdot E}{Q \cdot L \cdot 10^3}, \text{ min.}$$

NOTE: The purpose of adjusting sampling times is to obtain optimum fiber loading on the filter. A sampling rate of 1 to 4 L/min for 8 hrs (700 to 2800 L) is appropriate in non-dusty atmospheres containing ca. 0.1 fiber/mL. Dusty atmospheres require smaller sample volumes ($\leq$400 L) to obtain countable samples. In such cases take short, consecutive samples and average the results over the total collection time. For documenting episodic exposures, use high rates (7 to 16 L/min) over shorter sampling times. In relatively clean atmospheres, where targeted fiber concentrations are much less than 0.1 fiber/mL, use larger sample volumes (3000 to 10000 L) to achieve quantifiable loadings. Take care, however, not to overload the filter with background dust [6].

5. At the end of sampling, replace top cover and small end caps.
6. Ship samples upright with conductive cowl attached in a rigid container with packing material to prevent jostling or damage.
   NOTE: Do not use untreated polystyrene foam in the shipping container because electrostatic forces may cause fiber loss from sample filter.

SAMPLE PREPARATION:

7. Remove a circular section from any quadrant of each sample and blank filter using a cork borer [7].
8. Affix the circular filter section to a clean glass slide with a gummed page reinforcement. Label the slide with a waterproof marking pen.
   NOTE: Up to eight filter sections may be attached to the same slide.
9. Place the slide in a petri dish which contains several paper filters soaked with 2 to 3 mL acetone. Cover the dish. Wait 2 to 4 min for the sample filter(s) to fuse and clear.
   NOTE: The "hot block" clearing technique may be used instead of steps 8 and 9 [8, 9].
10. Place the slide containing the collapsed filters into a low-temperature plasma asher. Etch at 100 °C for ca. 2 min at an O_2 pressure of 130 Pa (1 mm Hg) [10].
    NOTE: Plasma ashers may vary. Determine optimum etching time (ca. 1/2 the time needed to completely ash a filter) on blank filters before etching samples.
11. Transfer the slide to a rotating stage inside the bell jar of a vacuum evaporator. Evaporate a 1- by 5-mm section of a graphite rod onto the cleared filter(s). Remove the slide to a clean, dry, covered petri dish [7].
12. Prepare a second petri dish as a Jaffe wick washer with the wicking substrate prepared from filter or lens paper placed on top of a 12-mm thick disk of clean, spongy polyurethane foam [11]. Cut a V-notch on the edge of the foam and filter paper. Use the V-notch as a reservoir for adding solvent.
    NOTE: The wicking substrate should be thin enough to fit into the petri dish without touching the lid.

13. Place the carbon-coated TEM grids face up on the filter or lens paper. Label the grids by marking with a pencil on the filter paper or by putting registration marks on the petri dish halves and marking with a waterproof marker on the dish lid. In a fume hood, fill the dish with acetone until the wicking substrate is saturated.
    NOTE: The level of acetone should be just high enough to saturate the filter paper without creating puddles.
14. Remove about a quarter section of the carbon-coated filter from the glass slide using a surgical knife and tweezers. Carefully place the excised filter, carbon side down, on the appropriately-labeled grid in the acetone-saturated petri dish. When all filter sections have been transferred, slowly add more solvent to the wedge-shaped trough to bring the acetone level up to the highest possible level without disturbing the sample preparations. Cover the petri dish. Elevate one side of the petri dish by placing a slide under it (allowing drops of condensed acetone to form near the edge rather than in the center where they would drip onto the grid preparation).

CALIBRATION AND QUALITY CONTROL:

15. Determine the TEM magnification on the fluorescent screen:
    a. Define a field of view on the fluorescent screen either by markings or physical boundaries.
       NOTE: The field of view must be measurable or previously inscribed with a scale or concentric circles (all scales should be metric) [11].
    b. Insert a diffraction grating replica into the specimen holder and place into the microscope. Orient the replica so that the grating lines fall perpendicular to the scale on the TEM fluorescent screen. Ensure that goniometer stage tilt is zero.
    c. Adjust microscope magnification to 10,000X. Measure the distance (mm) between the same relative positions (e.g., between left edges) of two widely-separated lines on the grating replica. Count the number of spaces between the lines.
       NOTE: On most microscopes the magnification is substantially constant only within the central 8 to 10 cm diameter region of the fluorescent screen.
    d. Calculate the true magnification (M) on the fluorescent screen:

$$M = \frac{X \cdot G}{Y}$$

       where: X = total distance (mm) between the two grating lines;
              G = calibration constant of the grating replica (lines/mm);
              Y = number of grating replica spaces counted
    e. After calibration, note the apparent sizes of 0.25 and 3.0 µm on the fluorescent screen. (These dimensions are essentially the diameter boundary limits for counting asbestos fibers by phase contrast microscopy.) Details for counting are given in step 21.
16. Measure 20 grid openings at random on a 200-mesh copper grid by placing a grid on a glass slide and examining it under the PCM. Use the Walton-Beckett graticule to measure the grid opening diameters. Calculate an average graticule field diameter from the data and use this number to calculate the graticule field area for an average grid opening.
    NOTE: A grid opening is considered as one graticule field.
17. Obtain reference selected area electron diffraction (SAED) or microdiffraction patterns from standard asbestos materials prepared for TEM analysis.
    NOTE: This is a visual reference technique. No quantitative SAED analysis is required [11]. Microdiffraction may produce clearer patterns on very small fibers or fibers partially obscurred by other material.
    a. Ensure that the specimen holder is oriented at zero tilt for diffraction pattern inspection.

b. Center a fiber, focus, and center the smallest field-limiting aperture on the fiber. Use a 20-cm camera length and 10X binocular head. Obtain a diffraction pattern. Photograph each distinctive pattern and keep the photo for comparison to unknowns.

NOTE: Not all fibers will present diffraction patterns. The objective lens current may need adjustment to give optimum pattern visibility. There are many more amphiboles which give diffraction patterns similar to the analytes named on p.7402-1. Some, but not all, of these can be eliminated by chemical separations. Also, some non-amphiboles (e.g., pyroxenes, some talc fibers) may interfere.

18. Acquire energy-dispersive X-ray (EDX) spectra on approximately 5 fibers having diameters between 0.25 and 0.5 μm of each asbestos variety obtained from standard reference materials [11].

NOTE: The sample may require tilting to obtain adequate signal. Use same tilt angle for all spectra.

a. Prepare TEM grids of all asbestos varieties.

b. Use acquisition times (at least 100 sec) sufficient to show a silicon peak at least 75% of the monitor screen height at a vertical scale of ≥500 counts per channel.

c. Estimate the elemental peak heights visually as follows:

(1) Normalize all peaks to silicon (assigned an arbitrary value of 10).

(2) Visually interpret all other peaks present and assign values relative to the silicon peak.

(3) Determine an elemental profile for the fiber using the elements Na, Mg, Si, Ca, and Fe. Example: 0-4-10-3-<1 [11].

NOTE: In fibers other than asbestos, determination of Al, K, Ti, S, P, and F may also be required for fiber characterization.

(4) Determine a typical range of profiles for each asbestos variety and record the profiles for comparison to unknowns.

MEASUREMENT:

19. Perform a diffraction pattern inspection on all sample fibers counted under the TEM, using the procedures given in step 17. Assign the diffraction pattern to one of the following structures:

a. chrysotile;

b. amphibole;

c. ambiguous;

d. none.

NOTE: There are some crystalline substances which exhibit diffraction patterns similar to those of asbestos fibers. Many of these, (brucite, halloysite, etc.) can be eliminated from consideration by chemistry. There are, however, several minerals (e.g., pyroxenes, massive amphiboles, and talc fibers) which are chemically similar to asbestos and can be considered interferences. The presence of these substances may warrant the use of more powerful diffraction pattern analysis before positive identification can be made. If interferences are suspected, morphology can play an important role in making positive identification.

20. Obtain EDX spectra in either the TEM or STEM modes from fibers on field samples using the procedure of step 18. Using the diffraction pattern and EDX spectrum, classify the fiber:

a. For a chrysotile structure, obtain EDX spectra on the first five fibers; one out of ten thereafter. Label the range profiles from 0-5-10-0-0 to 0-10-10-0-0 as "chrysotile."

b. For an amphibole structure, obtain EDX spectra on the first 10 fibers; one out of ten thereafter. Label profiles ca. 0-2-10-0-7 as "possible amosite"; profiles ca. 1-1-10-0-6 as "possible crocidolite"; profiles ca. 0-4-10-3-<1 as "possible tremolite"; and profiles ca. 0-3-10-0-1 as "possible anthophyllite."

NOTE: The range of profiles for the amphiboles will vary up to ±1 unit for each of the elements present according to the relative detector efficiency of the spectrometer.

c. For an ambiguous structure, obtain EDX spectra on all fibers. Label profiles similar to the chrysotile profile as "possible chrysotile." Label profiles similar to the various amphiboles as "possible amphiboles." Label all others as "unknown" or "non-asbestos."
NOTE: Fibers smaller than 0.2 µm in diameter may not produce sufficient peak heights to allow an elemental profile to be determined. Identify these fibers as "unknown".

21. Counting and Sizing:

a. Insert the sample into the specimen grid holder and scan the grid at zero tilt at low magnification (ca. 300 to 500X). Ensure that the carbon film is intact and unbroken over ca. 75% of the grid openings.

b. In order to determine how the grid should be sampled, estimate the number of fibers per grid opening during a low-magnification scan (ca. 1000X). This will allow the analyst to cover most of the area of the grid during the fiber count and analysis. Use the following rules when picking grid openings to count [11]:
(1) Light (<5 fibers per grid opening): count every grid opening in which the carbon film is intact.
(2) Moderate (5 to 25 fibers per grid opening): count every fifth grid opening. If the carbon film is damaged, proceed to the next available grid opening in which the film is intact.
(3) Heavy (>25 fibers per opening): count every tenth grid opening. If the carbon film is damaged, proceed to the next available opening in which the carbon film is intact.

c. Increase magnification to 10,000X. Begin counting at one end of the grid and systematically traverse the grid by rows, reversing direction at row ends. Count at least 2 field blanks per sample set to document possible contamination of the samples. Use the mean fiber count for the field blanks, B, in step 22. Count fibers and asbestos structures using the following rules:
NOTE: Microscopes which traverse nonlinearly or erratically, causing incomplete coverage of the grid opening are not suitable for asbestos analysis [12]. Before using a specific microscope for counting, it should be examined for accuracy and reproducibility of traverses.
(1) Count all particles less than 3 µm diameter which meet the definition of a fiber (aspect ratio ≥3:1, with parallel sides).
NOTE: Particles which are of questionable morphology should be analyzed by SAED and EDX to aid in identification.
(2) Size each fiber as it is counted and record the diameter and length (mm):
(a) Move the fiber to the center of the screen. Read the length of the fiber directly from the scale on the screen.
NOTE: For fibers which extend beyond the field of view, the fiber must be moved and superimposed upon the scale until its entire length has been measured.
(b) When a fiber has been sized, return to the starting point and continue the traverse to the next fiber.
(3) Record other asbestos structures according to the following morphological definitions. Label combinations of these structures according to the dominant quality.
(a) Bundle - compact arrangement of parallel fibers in which separate fibers or fibrils may only be visible at the ends or edges of the bundle.
NOTE: Asbestos bundles having aspect ratios of 3:1 or greater and less then 3 µm in diameter are counted as fibers.

(b) Cluster - network of randomly-oriented interlocking fibers arranged so that no fiber is isolated from the group. Dimensions of clusters can only be roughly estimated and clusters are defined arbitrarily to consist of more than four individual fibers [11].

(c) Matrix - one or more fibers attached to or embedded in a non-asbestos particle.

(4) Count fibers which are partially obscured by the grid.

NOTE: If a fiber is partially obscured by the grid bar at the edge of the field of view, count it as a fiber greater than 5 µm only if more than 2.5 µm of fiber is visible. Otherwise, log the fiber as a "short" fiber, measuring only that portion which is visible.

(5) When counting is complete, calculate the asbestos fiber fraction shorter than 5 µm and thinner than ca. 0.25 µm (the number of asbestos fibers which would be undetected by phase contrast microscopy).

d. Size all fibers using the scale on the fluorescent screen.

NOTE: Data can be recorded directly off the screen in mm and later converted to µm by computer. Count and record identified asbestos fibers and structures >1 µm long of all diameters [12]. However, when comparing TEM data to the PCM counts, all fibers, regardless of identification, greater than 5 µm long and between 0.25 and 3.0 µm diameter which have aspect ratios of 3:1 or greater should be included. This size adjustment is necessary to test comparability of counts between the two methods. If the size-adjusted counts show reasonable equivalency, then further analysis using the small-fiber data will show what fraction of airborne asbestos fibers is being missed by the PCM method.

CALCULATIONS:

22. Calculate and report fiber density on the filter, E, by dividing the total fiber count F, minus the mean field blank count, B, by the number of fields counted, n, (for each sample), and the field area, A_f.

$$E = \frac{\left(\frac{F}{n_f} - \frac{B}{n_b}\right)}{A_f}, \text{ fibers/mm}^2$$

NOTE: The field area, A_f, is considered to be one grid opening, and the size may vary depending upon the type of grids used. This value is known from step 16.

23. Calculate and report the average concentration, C, (fibers/mL) of fibers in the air sample, V (L), using the effective collection area of the filter, A_c (385 mm² for a 25-mm filter):

$$C = \frac{(E)(A_c)}{V \cdot 10^3}$$

24. As an integral part of the report, give the model and manufacturer of the TEM as well as the model and manufacturer of the EDS system.

EVALUATION OF METHOD:

The TEM method has been shown to have a precision of 0.275 (s_r) in an evaluation of mixed amosite and wollastonite fibers. The estimate of the asbestos fraction, however, had a precision of 0.11 (s_r). When this fraction was applied to the PCM count, the overall precision of the combined analysis was 0.20 [4].

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METHOD WRITTEN BY: James W. Carter; Paul A. Baron, Ph.D.; and David G. Taylor, Ph.D.; NIOSH/DPSE.

DRAFT

FORMULA: various

M.W.: various

ASBESTOS (bulk)
METHOD: 7403
ISSUED: 8/15/87

EPA Standard (Bulk): 1% (w/w)

PROPERTIES: solid, fibrous, minerals

SYNONYMS: actinolite [CAS #13768-00-8], grunerite (amosite) [CAS #12172-73-5] anthophyllite [CAS #17068-78-9], chrysotile [CAS #12001-29-5], crocidolite [CAS #12001-28-4], tremolite [CAS #14567-73-8].

SAMPLING	MEASUREMENT
BULK SAMPLE: 1 to 10 grams SHIPMENT: seal securely to prevent escape of asbestos SAMPLE STABILITY: indefinitely BLANKS: none required	TECHNIQUE: microscopy, polarized light with dispersion staining ANALYTE: actinolite, grunerite, anthophyllite, chrysotile, crocidolite, tremolite SAMPLE PREPARATION: depends upon type of sample under consideration EQUIPMENT: microscope, polarized light
ACCURACY	**RANGE: <1% to 100% asbestos (v/v)**
RANGE STUDIED: <1% to 100% asbestos	ESTIMATED LOD: to be determined PRECISION: to be determined

APPLICABILITY: Determination of percent asbestos minerals in bulk samples.

INTERFERENCES: Other fibers with optical properties similar to the asbestos minerals may be positive interferences. Optical properties may be obscured by coating on the fibers.

REAGENTS:

1. Refractive Index Liquids for Dispersion Staining: high-dispersion series, 1.550, 1.605, 1.620.
2. Refractive Index Liquids: 1.670 and 1.700.
3. Refractive Index Liquids: 1.490-1.570, 1.590-1.720 in increments of 0.002 or 0.004 (optional).
4. Asbestos reference samples.
5. Distilled Water (optional).
6. Concentrated HCl: ACS reagent grade (optional).

EQUIPMENT:

1. Sample containers: screw-top plastic vials of 10 to 50 mL capacity.
2. Microscope, polarized light, with polarizer, analyzer, port for wave retardation plate, 360° graduated rotating stage, substage condenser, lamp, lamp iris, and:
   a. Object lenses: 10X and 40X or near equivalent.
   b. Ocular lense: 10X minimum.
   c. Eyepiece reticle: crosshair.
   d. Dispersion staining object lens or equivalent.
   e. Compensator plate: 550 millimicron retardation.
3. Microscope slides: 75 mm x 25 mm.
4. Cover slips: 22 mm x 22 mm.
5. Ventilated hood or negative pressure glove box.
6. Mortar and pestle: agate or porcelain.
7. Microscope, binocular, ca. 10 to 45X.
8. Light source: incandescent or fluorescent.
9. Tweezers, dissecting needles, spatulas, probes, scalpels, and paper clips.
10. Glassine paper or clean glass plate.

SPECIAL PRECAUTIONS: Asbestos, a human carcinogen, should be handled only in a hood. [1]

SAMPLING:

1. Place several grams of the material to be analyzed in a sample container. (Sample should be between 1 and 10 grams).
   NOTE: For large samples (i.e., whole ceiling tiles) that are fairly homogenous, a representative small portion should be submitted for analysis.
2. Make sure containers are taped so they will not open in transit.
3. Ship the samples in a rigid container with sufficient packing material to prevent jostling or damage.

GROSS EXAMINATION:

4. Examine samples in the container visually and/or with the aid of a low magnification steromicroscope in a hood. Note the homogeneity of the sample. A preliminary estimate of fiber content of the larger fibers can be made at this time.
   NOTE: If necessary, a sample may be carefully removed from the container and placed on a glassine transfer paper or clean glass plate for examination.

SAMPLE PREPARATION:

5. In a hood, open container and with tweezers remove small portions of the sample which are as representative to obtain as possible.
   NOTE: a. If the sample appears to be slightly nonhomogeneous in the sample container, it can be mixed with a pair of tweezers or a screwdriver before taking the portion for analysis. If mixing in the sample container is not an adequate means to mix the sample, take small representative portions of each type of material and place on a glass slide.

b. If there are obvious separable layers, sample and analyze each separately.
c. If the sample has large, hard particles, it may be necessary to grind it in a mortar. Do not grind so fine that fiber characteristics are destroyed.
d. On hard tiles that may have thin inseparable layers, use a scalpel to cut through all the layers for a representative sample. The sample then can be cut into smaller pieces after placing refractive index oil on it before trying to smash it to a thinner thickness.
e. Other methods of sample preparation such as acid and sodium metaphosphate treatment and ashing are not normally necessary. However, if needed, they are described in Reference [2].

6. After placing a small portion of sample on the slide, put a few drops of 1.55 HD refractive index oil on the sample. Tease apart with a needle or smash small clumps with the flat end of a spatula or paperclip. (A uniform thickness of particles is desired so that better estimates of volume percentages can be made.) Mix the fibers and particles on the slide so that they are as homogeneous as possible.
NOTE: An even dispersion of sample should cover the entire area under the cover slip. Some practice will be necessary to judge the right amount of material to place on the slide. Too little sample may not give sufficient information and too much sample cannot be easily analyzed.

CALIBRATION AND QUALITY CONTROL:

7. Prepare a duplicate slide of at least 10% of the samples analyzed. Average the results for reporting.
8. Analyze about 5% blind samples of known asbestos content (e.g., from the EPA's Bulk Sample Analysis Quality Assurance Program).
9. If it appears that the preparation technique might not be able to produce a homogeneous or a representative sample on the slide, prepare a duplicate slide and average the results.
NOTE: Occasionally, when the duplicate results are greatly varying, it will be necessary to prepare additional replicate slides and average all the replicate results.
10. Follow the manufacturer's instructions for illumination and condensor alignment and other microscope adjustments.
11. Laboratories performing this analytical method should participate in the EPA's Bulk Sample Analysis Quality Assurance Program or a similar interlaboratory quality control program.
12. In order to perform this test method, an analyst should have completed a college-level course in mineralogy and/or had formal training in polarized light microscopy and its application to crystalline materials. In lieu of formal training for the analyst, a minimum of four months laboratory training in asbestos bulk analysis under the direction of a trained asbestos bulk analyst may be substituted.
NOTE: Due to the subjective nature of visual estimation of the asbestos content, an analyst needs to analyze samples on a frequent basis in order to remain proficient in estimating volume percentages.

ASBESTOS IDENTIFICATION AND QUANTITATION:

13. Scan the slide to identify any asbestos minerals using the optical properties of morphology, refractive indices, color, pleochroism, birefringence, extinction characteristics, sign of elongation, and dispersion staining characteristics.
NOTE 1: Techniques for determining these optical properties are described in References [3] through [8]. Optical properties for the asbestos minerals are listed in Table 1.
NOTE 2: There may be some differences from the standard characteristics due to natural variations in the conditions under which the minerals were formed or to which they have been subjected.

NOTE 3: In the 1.55 HD refractive index oil, chrysotile will be readily identifiable from mineral wool or fiberglass by crossing the polars and using the 550 millimicron retardation plate to observe the colors of chrysotile. Both of the glass species are isotropic and will not show any colors. Many varieties of cellulose are close to 1.55 in index, but will not show central dispersion staining colors. Characteristic magenta and blue colors identify chrysotile.

a. If the fibers in the sample have a higher index of refraction than 1.55, have a negative sign of elongation, and appear blue by transmitted light, crocidolite is suspected. Prepare another slide with 1.700 refractive index oil. The color of crocidolite will be much bluer with an annular stop. The central stop dispersion staining colors are sometimes difficult to impossible to see because of the opacity of the dark blue fibers.

b. If the fibers with the higher index than 1.55 are not blue, prepare a slide using 1.670 refractive index oil Amosite has a positive sign of elongation and in this oil has central stop dispersion staining colors of yellow and magenta-blue.

c. If the refractive index of the fibers is between 1.550 and 1.670, mount another preparation in 1.605 HD or 1.620 HD. The refractive indices for anthophyllite, tremolite, and actinolite vary naturally within the species. Anthophyllite can be distinguished from the other two by its parallel extinction. Actinolite has a light to dark green color with some pleochroism in transmitted light. The dispersion staining colors will have to be checked. A common interference mineral in this refractive index range is wollastonite. It also has a typical cleavage fragment morphology similar to the three asbestos minerals. Wollastonite has both a positive and a negative sign of elongation, parallel extinction, and central stop dispersion stain colors in 1.605 HD of pale yellow and pale yellow to magenta. If further confirmation of wollastonite versus anthophyllite is needed, wash a small portion of the sample in a drop of concentrated hydrochloric acid on a slide. Place the slide, with a cover slip in place, on a warm hot plate until dry. By capillary action, place 1.62 refractive index oil under the cover slip and then examine the slide. Wollastonite fibers will have a "cross-hatched" appearance across the length of the fibers and will not show central stop dispersion colors. Anthophyllite and tremolite will still show dispersion colors.

14. After the asbestos species have been identified, scan the entire area under the cover slip of the slide with the 1.55 HD refractive index oil and determine an estimated volume percentage of each asbestos mineral. The slides with the other index oils can be used to help confirm these estimates, if needed.

NOTE 1: Since complete homogeneity of the sample is difficult to obtain, a range in percentages may be preferable to estimate than to estimate a single percentage.

NOTE 2: If the estimate is in question, several microscopists can examine the slide and an average of their results then reported.

NOTE 3: Estimates of the volume percentages of the other fibers in the sample can also be determined and reported as required.

15. Make quantitative estimate of the asbestos content of the sample from the appropriate combination of the estimates from both the gross and microscopic examinations.

NOTE: Point counting techniques to determine percentages of the asbestos minerals is not generally recommended. The point counting method only produces accurate quantitative data when the material on the slide has a uniform thickness, which is difficult to obtain. The point counting technique is, however, recommended by the EPA to determine the amount of asbestos in bulk [2].

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METHOD WRITTEN BY: Patricia A. Klinger, CHIT and Keith R. Nicholson, CIH, DataChem, Inc., Salt Lake City, Utah, under NIOSH Contract _____.

Table 1. Optical Properties of Asbestos Fibers

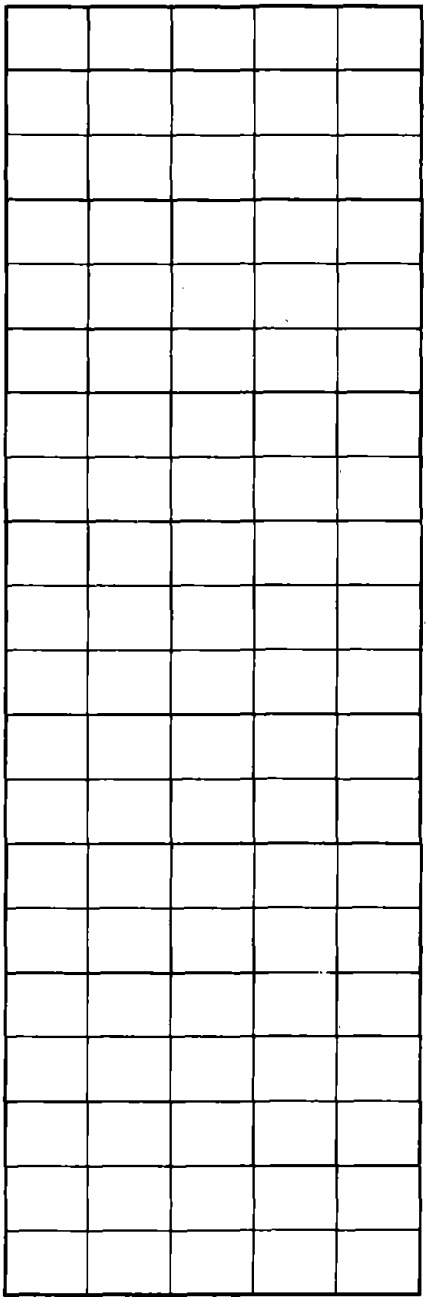
Mineral	Morphology and Color	Refractive Index (Approximate Values)		Birefringence
		$\parallel$ to Elongation	$\perp$ to Elongation	
Chrysotile Asbestos	Wavy fibers with kinks. Splayed ends on larger bundles. Colorless to light brown upon being heated. Nonpleochroic. Aspect ratio typically >10:1.	1.55	1.54	0.002 - 0.014
Cummingtonite-Grunerite Asbestos (Amosite)	Straight fibers and fiber bundles. Bundle ends appear broom-like or splayed. Colorless to brown upon heating. May be weakly pleochloric Aspect ratio typically >10:1.	1.70	1.67	0.02 - 0.03
Crocidolite Asbestos	Straight fibers and fiber bundles. Longer fibers show curvature. Splayed ends on bundles. Characteristic blue color. Pleochroic. Aspect ratio typically >10:1.	1.70	1.71	0.014 - 0.016 Interference colors may be masked by blue color.
Anthophyllite Asbestos	Straight fibers and fiber bundles. Cleavage fragments may be present. Colorless to light brown. Nonpleochroic to weakly pleochroic. Aspect ratio generally <10:1.	1.63	1.61	.019 - .024
Tremolite-Actinolite Asbestos	Straight and curved fibers. Cleavage fragments common. Large fiber bundles show splayed ends. Tremolite is colorless. Actinolite is green and weakly to moderately pleochroic. Aspect ratio generally <10:1.	1.62 - 1.64 (tremolite) 1.64 - 1.68 (actinolite)	1.60 - 1.62 (tremolite) 1.62 - 1.67 (actinolite)	0.02 - 0.03

Table 1. Optical Properties of Asbestos Fibers (Continued)

Mineral	Extinction	Sign of Elongation	Central Stop Dispersion Staining Colors: RI Liquid	⊥	∥
Chrysotile Asbestos	Parallel to fiber length	+ (length slow)	1.550 HD	Blue	Blue-magenta
Cummingtonite-Grunerite Asbestos (Amosite)	Parallel to fiber length	+ (length slow)	1.670	Red magenta to blue	Yellow
			Fibers subjected to high temperatures will not dispersion stain.		
Crocidolite Asbestos	Parallel to fiber length	- (length fast)	1.700	Red magenta	Blue-magenta
Anthophyllite Asbestos	Parallel to fiber length	+ (length slow)	1.605 HD	Blue	Gold to gold-magenta
			1.62 HD	Golden Yellow	Blue-green
Tremolite-Actinolite Asbestos	Oblique - 10 - 20° for fragments. Some composite fibers show ∥ extinction.	+ (length slow)	1.605 HD	Pale blue (tremolite)	Yellow (tremolite)
				Yellow (actinolite)	Pale yellow (actinolite)

Slide # _____ Field Area: _____ Name: _____

record your count
in vertical columns



Your Grade: _____

Your fib/mm^2: _____

Class fib/mm^2: _____

Class S_r (CV): _____

Times Counted: _____

Total count: _____ # Field observed: _____ Average count: _____

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