# **Fiber count** 243-1

| Standards (1) :   | Asbestos<br>Average permissible concentration:<br>0.2 fibers/cm <sup>3</sup> crocidolite and amosite;<br>1 fiber/cm <sup>3</sup> chrysotile or other asbestos.<br>Maximum permissible concentration :<br>1 fiber/cm <sup>3</sup> crocidolite and amosite;<br>5 fibers/cm <sup>3</sup> chrysotile and other asbestos. |
|-------------------|--|
| Sampling :        | Filter MCE, diameter filter : 25 mm;<br>Pore diameter : 0.8 to 1.2 $\mu$ m<br>Cassette: with conductive cowl<br>Flow rate : 0.5 to 16 L/min (0.5 to 2.5 L favored for average permissible<br>concentration in industrial environment)<br>Volume : minimum 400 L at 0.1 fiber/cm <sup>3</sup>                         |
| Analytical method | Fiber count by phase contrast light microscopy   |

# **Technical and scientific reports and notes** Analytical method 243-1

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ISBN: 2-550-20522-7 ISSN: 0820-8395

8 Institut de recherche en santé et en sécurité du travail du Québec, 1995

#### 1. Principle of the method

- 1.1 A known volume of air is aspirated across a mixed cellulose ester filter (MCE) to collect the fibers according to the method described in the sampling guide (1) and in this method (Appendix 1).
- 1.2 The filters are cleared and maintained in a refractive index environment less than or equal to 1.46 favouring fiber observation.
- 1.3 Fiber count is performed using an optical microscope with a phase contrast condenser with a magnification of approximately 400X.
- 1.4 In the context of convention no. 162 of the International Labor Organization, the terms « respirable asbestos dusts » target asbestos fibers whose diameter is less than 3 μm and the length-diameter ratio is greater than 3 :1. Only fibers that are longer than 5 μm will be considered for measurement purposes.

The term «asbestos» targets the fibrous forms of the mineral silicates belonging to metamorphic rocks of the serpentine group, namely chrysotile (white asbestos); and the amphibole group, namely actinolite, amosite (brown asbestos, cummingtonite-grunerite), anthophyllite, crocidolite (blue asbestos), tremolite ; or any mixture contai-ning one or more of these minerals.

1.5 This counting method applies also to counts of fibers other than asbestos whose refractive index is compatible with the mounting solution.

# 2. Field of application

- 2.1 The method's field of application corresponds to densities varying from 100 to 1,300 fibers/mm<sup>2</sup>. It is a function of volume sampled and the area of the counting field. Fiber densities from 25 to 100 fibers/mm<sup>2</sup>, which are less than the optimum densities, can be taken into consideration to evaluate the exposure of a worker ; however, the coefficient of variation of the method is not known at these densities.
- 2.2 Sensitivity

Fibers whose diameter is less than 0.25  $\mu$ m are not detected (2) by the method. The optimum upper limit can be increased by using a shorter sampling time or by decreasing the flow rate, whereas the lower limit of the field of application can be lowered by increasing the sampling volume.

#### 3. Interferences

Any other airborne fiber can interfere if it has the geometrical count criteria described in section 1.4. Furthermore, chain-like particles can be confused with fibers. High concentrations of non-fibrous particles can obscure fibers in the field of view and increase the method's lower limit of application.

# 4. Precision and accuracy

4.1 Coefficient of variation

In its official method #7400 A Rules, NIOSH reports a coefficient of variation (Sr) of 0.115 to 0.13 (3). An IRSST study carried out with 12 counters gave mean coefficients of variation of 0.28  $\pm$  0.08 for samples generated in the laboratory, and 0.34  $\pm$  0.12 for samples collected directly in industries (4).

- 4.2 Quality control
  - 4.2.1 Mixed cellulose ester filters
    - a) Filter verification

Quality control of mixed cellulose ester filters consists of checking each new lot of filters before it is used.

When a new lot of three to ten boxes is received, two boxes are checked. For a lot of ten to fifteen boxes, three boxes are checked. Take four filters per box of one hundred, or one per package of twenty-five. In each of the packages in a box, take the filter from a different section, namely from the first fourth, second fourth, etc., respectively.

Mount the filters according to the method described in section 7.1 of this document and count according to the criteria mentioned in section 7.2. These are considered as the laboratory blanks. Discard the lot of filters if the mean is greater or equal to 5 fibers per 100 graticule fields.

If one package out of four is not acceptable, again take one filter per package and check them. Again, if one filter out of four is not acceptable, the box is rejected. In the case of a lot of three to ten boxes where two boxes undergo quality control, if one box out of two is discarded, check a third one. Another discard leads to discarding the lot. In the case of a lot of eleven to fifteen boxes where three boxes undergo quality control, if one box out of three is rejected, then a fourth box is subjected to quality control ; discarding it leads to discard of the entire lot.

b) Field blanks

Prepare and count the field blanks (see Appendix 1) with the field samples. Note the results of each field blank. Calculate the average of the counts performed on the field blanks and subtract this value from each sample count.

- Note 1: The identity of the blanks must remain unknown to the counter until the counts have been completed.
- Note 2: If a field blank gives a result above 7 fibers/100 graticule fields, note a possible contamination of the samples.
- 4.2.2 Intra-laboratory quality control program for fiber counters
  - a) Document the precision of each laboratory counter by repetitive slide counts.

- 1) Maintain a series of reference slides to be used on a daily basis as part of the laboratory's quality assurance program. These slides should consist of filter preparations including a range of densities and levels of dust from various sources including samples generated in the laboratory and others sampled in the field. The person responsible for quality assurance must keep the reference slides and supply each counter with a minimum of one reference slide per workday. He should periodically change the labels on the reference slides so that the counters do not become familiar with the samples.
- 2) Without knowing the number of the sample, repeat the count on the reference slides and estimate the intra-laboratory and intercounter coefficient of variation, Sr. Obtain separate values of coefficients of variation for each matrix of samples analyzed in each of the following fields : 5 to 50 fibers in 100 graticule fields (6 to 65 f/mm<sup>2</sup>), 51 to 100 fibers in 100 graticule fields (66 to 125 f/mm<sup>2</sup>), and 100 to 500 fibers in 100 graticule fields (126 to 635 f/mm<sup>2</sup>). Keep quality control graphs for each of these data.
- b) Have random counts performed by the same counter on 10% of the filters counted (the slides are reidentified by a person other than the counter). Use the following test to determine whether a pair of counts by the same counter on one filter should be rejected because of possible bias : discard the sample if the difference between two counts exceeds 2.77(X)Sr, where X = the average of the two counts and Sr = the intra-counter standard deviation (step 4.2.2 or Appendix 2).
  - Note: If a pair of results is rejected by this test, recount the samples in this series and check the new results against the first results. Discard all rejected paired counts. It is not necessary to use these statistics on blanks.
- c) Enroll each new counter in a training course that compares the performance of counters on a variety of samples using this procedure.
- 4.2.3 Interlaboratory quality control program

All laboratories involved in counting fibers should participate in a quality control program such as the AIHA-NIOSH (PAT) Program and routinely exchange field samples with other laboratories to compare the performance of its counters.

The purpose of interlaboratory quality control is to evaluate the extent to which a count of one sample by a laboratory corresponds to the average count of a large number of laboratories. The discussion in Appendix 2 indicates how this estimate can be achieved on the basis of measurement of interlaboratory variability as well as by demonstrating how the results of this method are related to the possible theoretical count precision and to the interlaboratory and intra-laboratory coefficients of variation measured.

# 5. Equipment

- 5.1 Sampling : (see Appendix 1 for sampling details)
  - Three-piece, 25 mm cassette with 50 mm conductive extension cowl, a mixed cellulose ester filter whose pore size is 0.8 to 1.2 microns, and cellulose support.
    - Note: The use of an electrically conductive cowl reduces electrostatic effects. Ground the cowl when possible during sampling.
  - Appropriate personal sampling pump (1).
  - Multi-stranded 22 gauge wire.
- 5.2 Laboratory equipment
  - Positive phase contrast microscope, with green or blue filter, 8X to 10X eyepiece, and a 40X to 45X phase objective (approximate total magnification of 400X); numerical aperture of 0.65 to 0.75.
  - Glass slides, frosted-end, pre-cleaned, 25 mm x 75 mm.
  - Cover slips, 22 mm x 22 mm, No. 1-1/2, unless otherwise indicated by the microscope manufacturer.
  - Lacquer or nail polish.
  - Knife, #10 surgical steel, curved blade.
  - Tweezers.
  - Heated aluminum block for clearing filters on glass slides, or equivalent (5).
  - Micropipettes, 5, 100 and 500 µL.
  - Walton-Beckett graticule, type G-22, with a 100 µm diameter circular field (area = 0,00785 mm<sup>2</sup>) at the specimen plane.
    - Note: The graticule is specific to each microscope. Specify the disc diameter required to fit exactly the ocular of the microscope and the diameter (mm) of the circular counting area (see Appendix 3).
  - HSE/NPL phase contrast test slide, Mark II.

- Telescope, ocular phase-ring centering.
- Stage micrometer with 0.01 mm divisions.

#### 6. Reagents

6.1 Acetone

SPECIAL PRECAUTIONS:

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

6.2 Triacetin (glycerol triacetate), reagent grade.

# 7. Analytical protocol

- 7.1 Preparation of the sample :
  - 7.1.1 Ensure that the glass slides and cover slips are free of dust and fibers.
  - 7.1.2 Adjust the temperature of the heated block to approximately 70°C (5).
    - Note 1: If the hot block is not used in a fume hood, it must rest on a ceramic plate and must be isolated from any surface susceptible to heat damage.
    - Note 2: Other mounting techniques can also be used (for example, the procedure to generate acetone in the vapor form in a laboratory fume hood, as described in NIOSH method 7400, revision of May 15, 1985).
  - 7.1.3 Mount part of the sample filter on a clean glass slide.
    - 7.1.3.1 Cut wedges representing approximately 25% of the filter surface with the curved-blade surgical knife using a rocking motion to avoid tearing it. Place the wedge, dust side up, on the slide.
    - Note: Static electricity normally keeps the wedge on the slide.
    - 7.1.3.2 Insert the slide with sample into the opening at the base of the hot block. Place the end of a micropipette containing approximately 250  $\mu$ L of acetone in the inlet port at the top of the hot block. Inject the acetone into the vaporization chamber with a slow steady pressure on the plunger while holding the pipette firmly in place. After the filter clears (3 to 5 seconds), remove the pipette and the slide.
  - <u>CAUTION</u>: Even if the volume of acetone used is small, use safety precautions. Work in a well-ventilated area (for example a laboratory fume hood). Take care not to ignite the acetone. Frequent and continuous use of this instrument in an unventilated area can produce explosive concentrations of acetone vapour.

- 7.1.3.3 Using a 5  $\mu$ L micropipette, immediately place 3 to 3.5  $\mu$ L of triacetin on the wedge. Gently place a clean cover slip on the wedge at a slight angle in order to prevent the formation of air bubbles.
  - Note: If numerous air bubbles form or if the amount of triacetin is insufficient, the cover slip may become detached within a few hours. If an excess of triacetin remains at the edge of the filter under the cover slip, fiber migration may occur.
- 7.1.3.4 Glue the edges of the cover slip to the slide using lacquer or nail polish (6) once the filter has cleared.
- Note 1: If clearing is slow, heat the slide on a hotplate (surface temperature 50°C) for up to 15 minutes. Heat carefully to prevent the formation of gas bubbles.
- Note 2: Counting can begin immediately once clearing and mounting are completed.

# 7.2 Fiber count

7.2.1 Place the slide on the microscope stage and bring the center of the filter under the objective lens. Focus on the filter plane. Adjust the microscope according to step 8.1 (2).

Begin counting from the tip of the filter and progress outwards. Ensure that, as a minimum, each analysis covers one radial line from the filter center to the outer edge of the filter. Graticule fields are randomly selected by not looking in the eyepiece when advancing the mechanical stage.

- 7.2.2 Counting rules
  - 1) Count as a fiber any fiber meeting the following two counting criteria and which lies completely in the observed field :
    - Count only fibers whose diameter is less than 3 µm and that are longer than 5 µm. Measure curved fibers by taking into account their curvature to estimate the total length.
    - b) Count only fibers having a length-to-width (diameter) ratio greater than three.
  - 2) Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of the fiber.
  - 3) In the case where the fibers meeting rules 1 and 2 cross the graticule boundary :
    - a) Count as ½ fiber any fiber with only one end lying within the graticule area.

- b) Do not count any fiber that crosses the graticule boundary more than once.
- 4) Do not count all other fibers.
- 5) Count enough graticule fields to obtain 100 fibers. Count a minimum of 20 fields even if more than 100 fibers are counted. Stop counting at 100 fields even if 100 fibers have not been counted.
- 7.2.3 When a bundle covers 1/6 or more of a graticule field, reject the field and choose another. Do not report rejected fields in the total count.
- 7.2.4 When counting each graticule field, continuously scan a range of focal planes by moving the fine focus knob. This allows very fine fibers which have become embedded in the filter to be detected. Small-diameter fibers will be very faint but are an important contribution to the total count. A counting time of at least 15 seconds per field is appropriate for accurate counting.
- Note: This method does not allow for differentiation of fibers based on morphology. Although some experienced counters are capable of selectively counting different types of fibers, there is at present no accepted method that ensures uniformity of judgement between laboratories. It is therefore incumbent upon all laboratories using this method to report total fiber counts. Should serious contamination by fibers other than asbestos occur in the samples, other techniques such as transmission electron microscopy and polarized light microscopy must be used to identify the fraction of asbestos fibers present in the sample (see NIOSH methods 7402 and 7403) (7,8).

# 8. Calibration

8.1 Adjustment of the microscope

Follow the manufacturer's instructions. At least once a day, use the telescope ocular supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric. For each microscope, keep a logbook to record the microscope cleaning, adjustment and calibration dates.

- 8.1.1 Each time that a sample is examined, do the following :
  - 8.1.1.1 Adjust the light source for uniform illumination in the field of view at the condenser iris. With certain microscopes, illumination will have to be set up with bright field optics rather than phase contrast optics.
    - Note: Use Köhler illumination if available.
  - 8.1.1.2 Focus on the particulate matter to be examined.
  - 8.1.1.3 Ensure that the field iris is in focus, centered on the sample, and open only enough to completely illuminate the field of view.
- 8.1.2 Periodically check the microscope's detection limit for each analyst-microscope combination :
  - 8.1.2.1 Center the HSE/NPL phase contrast slide under the phase objective.
  - 8.1.2.2 Bring the series of grooved lines into focus in the graticule field.
  - Note: The slide contains seven blocks of grooves (approximately 20 grooves per block) in decreasing order of visibility. For asbestos counting, the microscope optics must completely resolve the grooved lines in block 3 even if they may appear somewhat faint, and the grooved lines in blocks 6 and 7 must be invisible when observed at the center of the graticule area. Blocks 4 and 5 must be partially visible and may vary slightly in visibility from one microscope to another. A microscope that cannot meet these criteria has too high or too low a resolution for fiber counting.
  - 8.1.2.3 If the quality of the image deteriorates, clean the microscope optics. If the problem persists, consult the manufacturer.
- 8.2 Calibration of the Walton-Beckett graticule

Using a micrometer divided into hundredths of a millimeter, regularly check the diameter of the graticule and ensure that the diameter remains within an acceptable range of 100  $\mu$ m ± 2  $\mu$ m. The corresponding surface is 0.00785 mm<sup>2</sup> ± 0.00032 mm<sup>2</sup>. To purchase a graticule, refer to Appendix 3.

#### 9. Calculations

9.1 Fiber density

Calculate the fiber density on the filter by dividing the total fiber count per graticule field (N/n), minus the mean field blank count per graticule field  $(N^t/n^t)$ , by the graticule field area A, (0.00785 mm<sup>2</sup> for a properly calibrated Walton-Beckett graticule) :

$$E = \frac{\left[\frac{N}{n} - \frac{N^{t}}{n^{t}}\right]}{A}$$

where E = fiber density (fibers/mm<sup>2</sup>)

- N = total fiber count for the sample
- n = number of fields observed for the sample
- N<sup>t</sup> = average number of fibers on the blank filter
- n<sup>t</sup> = number of fields observed for the blank filter
- A = graticule area
- Note: Fiber counts above 1,300 fibers per mm<sup>2</sup> and fiber counts from samples with more than 50% of the filter area covered with particles should be reported as « uncountable » or « probably biased ».
- 9.2 Concentration

Calculate the concentration of fibers in the air volume sampled, using the effective collection area of the filter, (385 mm<sup>2</sup> for a 25 mm filter):

$$C = \frac{(E)(a)}{(d)(t)(1000)}$$

where  $C = \text{concentration (fibers/cm}^3)$ 

- E = fiber density (fibers/mm<sup>2</sup>)
- a = effective collection area of the filter (mm<sup>2</sup>)
- d = flow rate (L/min)
- t = sampling time (min)

Note : Periodically check that the value of « a » does not change.

#### 9.3 Reporting of results

Report intra-laboratory and interlaboratory coefficients of variation Sr (from step 4.2.2) with each set of results.

Note: Precision depends on the total number of fibers counted (3,9). Relative standard deviation (also called the coefficient of variation) is documented in references (3,9,10,11) for fiber counts up to 100 fibers in 100 graticule fields. Comparability of interlaboratory results is discussed in Appendix 2. 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, Appendix 2).

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#### Appendix 1

# Sampling

- 1. Adjust the flow rate of each personal sampling pump with an appropriate instrument (1).
- 2. For personnal sampling, attach the sampler to the worker near his breathing zone. Remove the cover from the cowl extension and orient face down. Wrap shrink tape between the extension and the cassette to avoid air leaks.
  - Note: If possible, ground the cassette to eliminate any surface charge by using a wire connecting the conducting extension to a non-electrical metal fixture such as a cold water pipe or a steel beam.
- 3. Submit for each series of samples at least two blanks or 10% of the total samples, whichever is greater.
- 4. Sample at 0.5 L/min or more (12). Adjust the sampling flow rate, d (L/min) and the time t (min) in such a way as to obtain a fiber density, E, of 100 to 1,300 fibers/mm<sup>2</sup> on a 25 mm filter (effective collection area of 385 mm<sup>2</sup>) to obtain optimum precision.

$$t = \frac{(a)(E)}{(d)(L)\,1000}$$

Note: The purpose of adjusting the sampling time is to obtain optimum fiber loading on the filter. A sampling rate of 1 to 4 L/min over a period of 8 hours is appropriate in non-dusty atmospheres containing for example 0.1 fibers per mL. Dusty atmospheres require smaller sampling volumes (less or equal to 400 L) to obtain countable samples.

In such cases, take short consecutive samples. Determine the average concentration as stated in the regulation S-2.1, r.15 (13). In order to document episodic exposure, use high flow rates (7 to 16 L/min) with shorter sampling times. In relatively clean atmospheres, where the concentration of fibers is much lower than 0.1 fibers/mL, use larger sampling volumes (3,000 to 10,000 L) in order to obtain quantifiable densities. Take care however, not to overload the filter with dust. If a filter surface greater or equal to 50% is covered with particles, the filter may be too overloaded to count and will lead to a bias in the measured fiber concentrations.

- 5. At the end of sampling, replace the covers and end caps.
- 6. Ship the samples with the conductive cowl attached in a rigid container in order to avoid jostling or damage.
  - Note : Do not use untreated polystyrene foam as a shipping container because electrostatic forces may result in fiber losses from the filter surface.

#### **APPENDIX 2**

#### INTERLABORATORY COMPARABILITY

Theoretically, the process of counting fibers distributed randomly (Poisson) on the filter surface gives an Sr that depends on the number, N, of fibers counted :

$$Sr = \frac{l}{N^{1/2}}$$

Sr is therefore 0.1 for 100 fibers and 0.32 for 10 fibers counted. The actual Sr found in a number of studies is greater than these theoretical values (9,10,11,14).

An additional component of variability comes from subjective differences between laboratories.

In a study involving 10 counters in a continuing sample exchange program, Ogden (9) found that this intra-laboratory subjective component is approximately 0.2 and he estimated the overall Sr by the expression :

$$Sr = \frac{[N + (0, 2 \times N)^2]^{1/2}}{N}$$

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

In a study on field results in 46 laboratories, the Asbestos Information Association (AIA) (11) also observed that the variability has a constant component as well as a component that depended on fiber count. These results have given a subjective interlaboratory component of the coefficient of variation Sr for the field samples of approximately 0.45 (on the same basis as Ogden's). A similar value was obtained from 12 laboratories analyzing a series of 24 samples (15). This value is slightly higher than the range of Sr (0.25 to 0.42 for 1984-85) found for 80 reference laboratories in the NIOSH PAT program for laboratory-generated samples (10).

Several factors influence the value of Sr for a given laboratory, such as the actual counting performance of a laboratory and the types of samples to be analyzed. In the absence of other information, such as interlaboratory quality assurance programs using field samples, the value of the subjective component of variability used is 0.45. It should be noted that, although based on two studies, it is a somewhat arbitrary choice. It is hoped that by using this value in the absence of additional information, laboratories will carry out the recommended interlaboratory quality assurance programs to increase their performance and reduce their Sr.

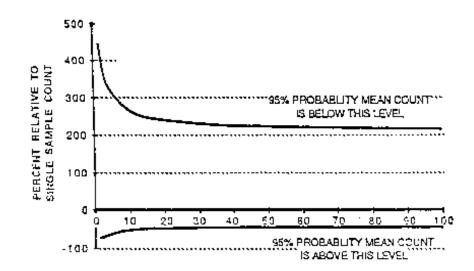
The relative standard deviations (Sr) described above apply when the mean of the population has been determined. However, it is more appropriate for laboratories to estimate a 90% confidence interval on the mean fiber count from a single sample (Figure 1). These curves assume similar shapes of the count distribution for interlaboratory and intra-laboratory results (5).

For example, if a sample gives a count of 24 fibers, Figure 1 indicates that in 90% of the cases, the mean interlaboratory count will fall within the range of 227% above and 52% below this value. These percentages can be applied directly to the concentrations in the air. If for example, this sample (24 fibers counted) represents a volume of 500 liters, then the measured concentration is 0.02 fibers/mL (assuming 100 fields counted, a 25 mm filter and a 0.00785 mm<sup>2</sup> 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 fibers/mL. These limits should be reported in any comparison of results between laboratories.

It should be noted that the Sr of 0.45 used to calculate Figure 1 is considered as an estimate for a random group of laboratories. If several laboratories belonging to a quality insurance group can show that their interlaboratory Sr is smaller, then it is more correct to use the smallest Sr. It has been found that Sr can be greater for certain types of samples, such as asbestos cement (14).

One can see from Figure 1 that the Poisson component of the variability is not very important unless the number of fibers counted is small. As a result, an acceptable approximation consists of simply using +213% and -49% as the upper and lower confidence limits of the mean for a count of 100 fibers.





# NUMBER OF FIBERS COUNTED IN A SINGLE SAMPLE FIGURE 1 : INTERLABORATORY PRECISION

#### **APPENDIX 3**

### CALIBRATION OF THE WALTON-BECKETT GRATICULE

Before ordering a Walton-Beckett graticule, the following calibration must be carried out in order to obtain a counting area (D) 100  $\mu$ m in diameter at the image plane. The diameter d<sub>c</sub> (mm) of the circular counting area and the diameter of the disc must be specified when ordering the graticule.

- 1) Insert any available graticule in 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 in such a way that magnification remains constant.
- 3) Install the 40x 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 length of the magnified graticule grid,  $L_o$  ( $\mu$ m), using the stage micrometer.
- 6) Remove the microscope graticule and measure its actual grid length, L<sub>a</sub> (mm). This can best be accomplished using a stage equipped with verniers.
- 7) Calculate the diameter of the circle,  $d_c$  (mm), for the Walton-Beckett graticule:

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

Example: If  $L_0 = 112 \mu m$ ,  $L_a = 4,5 mm$  and  $D = 100 \mu m$ , then  $d_c = 4,02 mm$ .

Check the field diameter, D (acceptable range of  $100 \,\mu\text{m} \pm 2 \,\mu\text{m}$ ) with the micrometer upon receipt of the graticule from the manufacturer. Determine the field area (acceptable range of 0.00785 mm<sup>2</sup> ± 0.00032 mm<sup>2</sup>).

#### **APPENDIX 4**

# **EXAMPLES OF COUNTING RULES**

Figure 2 shows a Walton-Beckett graticule as it is seen through a microscope. Even if the graticule incorporates the 3 :1 aspect, the counting rules will be discussed since they apply to the fibers identified in Figure 2.

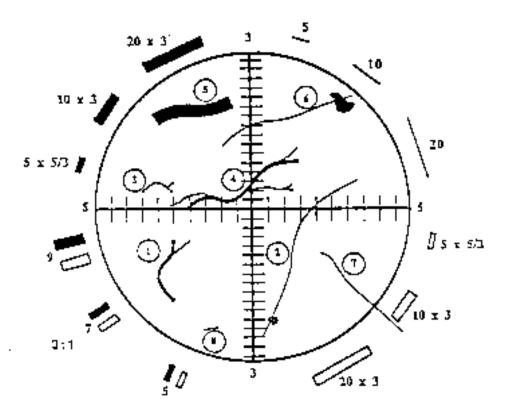


FIGURE 2 : WALTON-BECKETT GRATICULE WITH FIBERS

# FIBER COUNT

| Fiber number | <u>Count</u> | Discussion   |
|--------------|--------------|--|
| 1            | 1 fiber      | The criteria do not allow for split ends ; as a result, count one fiber;   |
| 2            | 1 fiber      | Single fiber with attached particle. The particle is treated as though it does not exist.  |
| 3            | 1 fiber      | As for fiber 1, count one fiber because it meets the >3 :1, > 5 $\mu$ m in length criteria.  |
| 4            | 1 fiber      | All fiber ends are attached to a large central fiber or bundle ; as a result, count one fiber.   |
| 5            | 0 fiber      | The fiber has a diameter greater than 3 $\mu m.$   |
| 6            | 1 fiber      | Ignore non-fibrous particulate matter ; count this as a whole fiber.   |
| 7            | 2 fiber      | Fibers that meet criteria 7.2.2.a and 7.2.2.b and that cross<br>the graticule boundary are counted as half fibers provided<br>the fiber does not cross the graticule boundary more than<br>once. In such a case, the fiber is not counted, regardless of<br>the number of ends that lie within the graticule area. |
| 8            | 0 fiber      | The fiber is shorter than 5 $\mu m.$   |