
**Air quality — Determination of the
number concentration of airborne
inorganic fibres by phase contrast
optical microscopy — Membrane filter
method**

*Qualité de l'air — Détermination de la concentration en nombre de
fibres inorganiques en suspension dans l'air par microscopie optique
en contraste de phase — Méthode du filtre à membrane*

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 146, *Air quality*, Subcommittee SC 2, *Workplace atmospheres*.

This second edition cancels and replaces the first edition (ISO 8672:1993), which has been technically revised. This second edition provides additional quality assurance procedures.

Introduction

The concentration of optically visible airborne inorganic fibres can only be defined in terms of the results obtained with a particular measurement method. Moreover, experience has shown that different laboratories, using the membrane filter optical counting method, can obtain different results on the same sample, even when the laboratories appear to be working from a written version of the method which attempts to specify all variables.

Because of the unusual operator-dependence of the membrane filter method, it is important to apply this method with care and use it in conjunction with a quality control scheme. The second edition of this International Standard provides for additional quality assurance procedures.

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Air quality — Determination of the number concentration of airborne inorganic fibres by phase contrast optical microscopy — Membrane filter method

1 Scope

This International Standard specifies the determination of the number concentration of airborne inorganic fibres by phase contrast optical microscopy using the membrane filter method in workplace atmospheres, as defined by the counting criteria given in [6.5.4](#).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 13137, *Workplace atmospheres — Pumps for personal sampling of chemical and biological agents — Requirements and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

NOTE Terms specific to this document are defined, in addition to those found in ASTM Standards D7200-12, [\[6\]](#) European Standard EN 1540. [\[7\]](#)

3.1

reference slide

slide prepared from a field sample by the acetone-triacetin method ([Annex A](#)) or the dimethyl formamide-Euparal method ([Annex B](#)) with a non-gridded cover slip that is to be used in a long-term quality control scheme

Note 1 to entry: For the inventory of reference slides, they should be selected from a previous prepared bank of samples for which the mean and variability have been historically established. They should also comprise of samples with varying fibre densities, and if available different fibre types. Reference slides should be checked for filter integrity periodically and replaced if necessary.

3.2

breathing zone

space around the nose and mouth from which a worker's breath is taken

Note 1 to entry: Technically, the breathing zone corresponds to a hemisphere (generally accepted to be a 30 cm in radius) extending in front of the human face, centred on the mid-point of a line joining the ears. The base of the hemisphere is a plane through this line, the top of the head and the larynx. This technical description is not applicable when respiratory protective equipment is used. [\[7\]](#)

3.3

countable fibre

any object having a maximum width less than 3 μm , an overall length greater than 5 μm and a length to width ratio greater than 3:1.

3.4 occupational exposure limit value

limit of time-weighted average of the concentration of a chemical agent in the air within the breathing zone of a worker in relation to a specified reference period

Note 1 to entry: Limit values are mostly set for reference periods of 8 h, but can also be set for shorter periods or concentration excursions. Limit values for gases and vapours are stated in terms independent of temperature and air pressure variables in ml/m³ and in terms dependent on those variables in mg/m³ for a temperature of 20 °C and a pressure of 101,3 kPa. Limit values for airborne particles and mixtures of particles and vapours are given in mg/m³ or multiples of that for actual environmental conditions (temperature, pressure) at the workplace. Limit values of fibres are given in numbers of fibres/m³ or number of fibres/cm³ for actual environmental conditions (temperature, pressure) at the workplace.^[7]

4 General method description

A sample is collected by drawing a measured quantity of air through a membrane filter by means of a battery-powered sampling pump. The entire filter or a portion of the filter (wedge) is later transformed from an opaque membrane into a homogeneous optically transparent specimen. The fibres are then sized and counted using a phase contrast optical microscope. The result is expressed as fibres per cubic centimetre of air, calculated from the number of fibres on the filter and the measured volume of air sampled. The method is applicable for routine sampling and sample evaluation necessary to assess personal exposure to fibres and implement control measures of their presence in occupational environments. The method is applicable for routine static sampling and measurement of personal exposure to fibres.

4.1 Limitations of the method by particle type

This method cannot identify the composition or characteristics of particular fibre types and its use shall be restricted to workplace atmospheres where the predominant fibre types are inorganic.

The use of this method also has limitations when applied to samples containing platy or acicular particles and consequently it should not be implemented without prior knowledge of the fibres present in the workplace atmosphere. There are a variety of analytical methods which can be useful, e.g. polarizing light microscopy, electron microscopy.

4.2 Limit of visibility and detection limits

This procedure cannot enumerate thin fibres whose width is below the limit of visibility by phase contrast optical microscopy. The limit varies according to the refractive index contrast between the fibres and the mounting medium, and the phase-shift of the microscope. The triacetin mounting medium proposed in this method has a refractive index of approximately 1,45, and the Euparal mounting medium has a refractive index of 1,48. In workplace atmospheres, fibres with refractive indices in the range of 1,4 to 1,5 might occur. As the relatively small refractive index difference between these fibres and the mounting media might not be sufficient for them to be visible, this mounting media might not be appropriate.

Previously published method limitations of 0,2 µm or 0,25 µm width limits are conservative consensus values. Practical studies have indicated the ability of a microscope properly adjusted to detect chrysotile fibres of 0,15 µm width^[11] and Amosite fibres of 0,062 5 µm width.^[12] These results suggest crocidolite fibres can be detectable at 0,05 µm width. Fibres with smaller widths can be detected under the electron microscope, but large differences in results sometimes observed between the two methods are more likely due to undercounting fine fibres under phase contrast microscopy (PCM) than to the presence of substantial numbers of fibres that can only be seen under the electron microscope. The quality assurance procedures in this International Standard are used to identify and resolve several types of counting errors under PCM.

With the parameters specified in this method, the theoretical lower detection limit for a sample of 480 l of air is 0,007 fibres/cm³. However, the limit of practical use is often 0,1 fibres/cm³ or higher. This is because blank filters can frequently give a reading of several countable fibres per 100 graticule areas. These "fibres" are contaminants on the filter, or artefacts from the clearing process which have the

appearance of fibres. Neither counting more fields nor increasing sampling duration overcomes the problem of background dust, when fibres are a minor constituent of the dust cloud. In relatively clean atmospheres, such as cleaned enclosure after asbestos removal (clearance sampling), the expected fibre concentration is $< 0,01$ fibres/cm³, larger sample volumes (>480 l) are required to achieve quantifiable loadings.

4.3 Apparatus and equipment

4.3.1 Sampling equipment

4.3.1.1 Filters. Membrane filters (mixed cellulose ester or cellulose nitrate) of 0,8 to 1,2 μm or less pore size and a diameter of 25 mm are preferred with, or without printed grids (printed grids can allow the counter to focus easier on the plane containing the fibres, but the lines of the grid can obstruct all or parts of the fields of view and interfere with the counting so that these fields must be avoided).

In recent years, problems have been observed with portions of batches of mixed cellulose ester filters, where the porosity is not evenly developed over the filter. Areas of the filter without porosity can lead to a high pressure drop resulting in premature pump failure, areas of the filter without fibres deposited, and the appearance of cracking in acetone-triacetin mounts.^[13] It is necessary to pay attention to the quality of filters in order to avoid these problems. In addition, each batch of filters should be tested for fibrous contamination as described in 5.4.

4.3.1.2 Open-faced filter holder fitted with a protective cowl.^[14]

The distance between the cowl opening and the filter plane should be between one and a half times and two times the internal diameter of the cowl. The internal diameter of the cowl should be at least equal to the exposed diameter of the filter but not more than 2 mm greater.

The cowl helps to protect the filter from accidental contamination. A conducting cowl is preferred to a plastic one because of the possible risk of fibre loss due to electrostatic charge. Filter holders and cowls shall be thoroughly washed before re-use.

Due to the design of the filter support utilized in some filter holders, a supporting pad should be used. The purpose of this supporting pad is to ensure an even distribution of air passing through the primary membrane.

4.3.1.3 Sampling pump, capable of giving a smooth flow and having flow set to within ± 5 % of the required flowrate, and of maintaining this flowrate through the filter to within ± 10 % for flowrate 2 l/min and ± 5 % for flowrate > 2 l/min during the period of sampling.

Although some pumps are equipped with pulsation dampers, an external damper might have to be installed between the pump and the collecting media. Personal sampling pumps shall meet the criteria for a Type P pump as detailed in ISO 13137.

4.3.1.4 Connecting tubing, constriction-proof and the connections shall be leak-proof.

4.3.2 Microscope equipment

Because microscopes with identical "specifications" can give quite different performances, it is necessary that the performance of the proposed and existing microscopes be assessed by means of a detection limit test slide. Provided this criterion is met, small departures from the recommended specifications in items 4.3.2.4 and 4.3.2.5 are permitted. The necessary specifications are as follows.

4.3.2.1 Light source-Kohler or Kohler type illumination. It is preferable for the illuminator to be built-in with a variable light intensity control.

4.3.2.2 Substage assembly, Abbe or achromatic phase contrast condenser incorporated into a substage unit.

There shall be a means of centering each condenser annulus with respect to the phase plate in the corresponding objective, and also a means of focusing the condenser.

4.3.2.3 Stage, a built-in mechanical specimen stage fitted with slide clamps and x-y displacement.

4.3.2.4 Objectives, a rotating nose-piece fitted with 10X and 40X parfocal phase contrast achromatic objectives.

The 40X objective shall have a numerical aperture (NA) between 0,65 and 0,75. It shall have a phase ring of absorption not less than 65 % and not greater than 85 %.

4.3.2.5 Binocular eyepieces, chosen to give a total magnification of 400X to 500X.

At least one eyepiece shall permit the insertion of a graticule. The compensating and focusing type is recommended.

4.3.2.6 Graticule (Walton-Beckett or RIB),^[15] the diameter of the graticule in the object plane, when using the 40X phase objective and an appropriate eyepiece, shall be $100 \pm 2 \mu\text{m}$.

4.3.3 Accessories

4.3.3.1 Centering telescope or Bertrand lens, for checking that the phase rings in the condenser are centred with respect to those in the objectives.

4.3.3.2 Green filter, to ensure the best phase contrast conditions because the optics are designed for this wavelength.

4.3.3.3 Stage micrometer, with 1 mm divided into 0,01 mm divisions.

4.3.3.4 Scalpel holder and Disposal blades, #10 or #22 surgical steel, curved blade.

4.3.3.5 Tweezers, fine point.

4.3.3.6 Acetone vaporizer, to clear mixed cellulose filters.

4.3.3.7 Hypodermic syringe, with 22 gauge needle or disposable micropipette.

4.3.3.8 Pre-cleaned microscope slides, of approximately 76 mm x 25 mm and 0,8 to 1,0 mm thick.

4.3.3.9 Cover slips (without grids), 22 mm x 22 mm, 0,16 to 0,19 mm thick, e.g. No. 1-1/2 or as specified by microscope manufacturer. Larger cover slips are necessary to cover a whole 25 mm diameter filter.

4.3.3.10 Phase contrast test slide, HSE/NPL Mark II or HSE/ULO Mark III where the certificate includes reference to at least one block of lines that should not be visible (see [6.4](#)).

4.3.3.11 Relocatable cover slips, each cover slip has 2 grids and 2 logos which help to orient the cover slip.

Each grid has 140 viewing fields, each of which is approximately 100 μm in diameter. The viewing fields are arranged into 14 columns and 10 rows. With proper orientation, a letter appears on the top and

bottom of each column and a number appears on either side of the rows. Thus, each viewing field is identified for relocation.^[9]

4.3.3.12 Standard relocatable test slides, prepared from different types of asbestos and inorganic fibres with different matrix background by the dimethyl formamide/ Euparal method.^[9]

There should be no fibre migration observed in these slides for more than 5 years.^[8] Other clearing and mounting procedures can be used if no filter migration is observed over the term of use.

They can be prepared from a proficiency test filter from the Proficiency Analytical Testing program (PAT) of the American Industrial Hygiene Association's (AIHA) Laboratory Quality Programs.^{[8][9]} The filter or filter wedge is cleared and mounted by the dimethyl formamide-Euparal method with a relocatable gridded cover slip ([Annex B](#)). The fibres visible in each grid opening have been identified and their locations marked on a drawing of each opening. The identity, number and position of each fibre have been verified by a second counter.

4.3.3.13 Disposal gloves.

4.3.3.14 Thermostat-control hotplate or drying oven.

4.3.3.15 Thermometer, 0 °C to 100 °C.

4.3.4 Reagents

4.3.4.1 Dimethyl formamide, reagent grade.

4.3.4.2 Glazier acetic acid, reagent grade.

4.3.4.3 De-ionized water.

4.3.4.4 Euparal resin.

4.3.4.5 Acetone, reagent grade.

4.3.4.6 Triacetin, reagent grade.

4.3.4.7 Lacquer or nail polish.

4.4 Mounting media

Acetone-triacetin is the mounting medium most often used (see [Annex A](#)). However, fibre migration can occur over time when excess triacetin is used. While this does not affect the analysis of routine samples and it might not affect the count concentration over time, it does restrict the ability to perform quality checks by re-examining the same areas. This problem can be controlled by using an appropriate amount of triacetin. However, the visual quality of the slides made with triacetin also deteriorates in about 12 months. Therefore, for permanent slides, the dimethyl formamide-Euparal mounting method (see [Annex B](#)) should be used. No fibre migration or visual quality deterioration has been observed in slides more than 5 years old.^[8] Fibre counts are not affected by using Euparal in place of triacetin,^[9] ^[16] and have also been shown to be equivalent to fibre counts using the dimethyl phthalate-diethyl oxalate method which was used previously for samples that were instrumental in the development of risk assessments.^[16]

4.5 Quality assurance

Subjective recognition and counting of fibres results in greater uncertainty compared to other analytical procedures so that particular attention must be paid to quality control measures. Slides are available, made from proficiency test filters from the PAT of the AIHA's Laboratory Quality Programs.^{[8][9]} Each slide has been mounted with a permanent Euparal medium and covered with a relocatable gridded cover slip. The fibres visible in each grid opening have been identified and their locations marked on a drawing of each opening. The identity, number and position of each fibre has been verified by a second counter. These slides are referred to in this International Standard as standard relocatable test slides, and they are applied in various ways to improve and assess the quality of fibre counts. The laboratory should also maintain an inventory of reference slides, being slides of field sample with varying fibre densities (and, if available, different fibre types). The labels on the reference slides and standard test slides are changed periodically so that the counter does not become familiar with the slides. The following quality control measures shall be applied on each day that field samples are counted:

- a) Examination of a standard relocatable test slide. The fibres in each designated field are counted and the counts referred to the accompanying slide descriptions. Counters shall obtain a discrepancy score of more than 50 before proceeding (6.4). If a score of 50 or better is not achieved, the counter should review the slide descriptions to determine the cause and then attempt to rectify the situation by repeating the microscope set-up or re-training the counter.
- b) Counting of a reference slide.
- c) Re-counting of 10 % of sample slides. Prior to re-counting, the slide is relabeled by a person other than the counter.

Prior to counting field samples, all counters should be trained in the techniques contained in this International Standard. Documentation of such training shall be maintained by the counter or laboratory. Training on both chrysotile and Amosite is the minimum requirement for asbestos analysis. Completion of successful training shall be documented through the analysis of standard relocatable test slides with a discrepancy score greater than 50 in each.

The following test is performed to determine whether a pair of counts on the same filter should be rejected because of possible bias. The sample is to be discarded if the absolute value of the difference between the square roots of the two counts (fibres/mm²) exceeds $2,77(X)S'_r$, where X = average of the square roots of the two fibre counts (fibres/mm²) and S'_r = half of the intracounter relative standard deviation, which should be derived by each laboratory based on the analyses of reference slides.^[17]

Further elements of quality control, such as microscope set-up and calibration are detailed under 6.2 to 6.3. Additional quality control measures such as participation in a proficiency test program, where available, and sharing slides with other laboratories to compare counting might also be useful.

5 Sampling

5.1 Flowrate

For workplace atmospheres, the flowrate for personal sampling shall be adjusted to approximately 0,5 l/min to 16 l/min. The adjustment of sample density to the range specified in 5.2 should be done by adjusting sampling time as in 5.5. The flowrate shall be checked at least before and after sampling. If the difference from the initial flowrate is >10 % for flowrate 2 l/min and 5 % for flowrate >2 l/min, the sample shall be rejected. If an external flowmeter is used to determine the flowrate of the pump, care shall be taken to ensure that the flowmeter does not cause unknown changes to the flowrate. Measurements of the "sampling train" flowrate using a soap-film flowmeter with and without the external flowmeter, is one satisfactory method of determining any change in flowrate. The flowmeter used shall be able to measure flowrate to an accuracy within ± 5 % of the true flow (95 % confidence limit).

For low concentration sampling, flowrates and sampling times are adjusted to obtain optimum fibre loading on the filter. The collection efficiency does not appear to be a function of flowrate in the range of 0,5 l to 16 l/min for asbestos fibres.^[18] However, it has been shown that the counts produced might be

a function of fibre density such that fibre densities below 100 fibres/mm² can be subject to bias,^[19] and that even in the range 100 fibres/mm² to 1 000 fibres/mm² samplers operating at different flowrates can give different counts.^[20]

For clearance sampling, the air should be relatively fibre free so that it is nearly impossible to collect the recommended fibre loading on the filter (5.2). The flowrates and sampling times are adjusted to collect enough sampling volumes to get to the desired fibre densities or required limit of detection (Tables 1 and 2).

5.2 Acceptable fibre loadings on filters

5.2.1 Minimum loading

The minimum filter loading should be greater than 100 fibres/mm² (i.e. approximately 0,8 fibres/Walton-Beckett graticule field). In special circumstances (e.g. in clearance sampling), it is permissible to lower the acceptable fibre loading to 20 fibres/mm² (i.e. approximately 0,15 fibres/Walton-Beckett graticule field).

The lowering of the acceptable fibre loading gives, at best, barely acceptable coefficients of variation. The limitations described in 4.2 should also be considered when measuring very low fibre concentrations.

5.2.2 Maximum loading

The filter loading should not exceed a maximum of approximately 650 fibres/mm² (5 fibres/graticule field averaged for all counted fields) for the majority of sampling situations. This might need to be reduced to an average of about one fibre per graticule field when mixed dusts or agglomerates are present, and can sometimes be doubled when only fibres are present. Average filter loadings exceeding 5 fibres / graticule field tend to result in an under estimation and should be treated with caution.

5.3 Storage and transport

Filters should be transported in closed holders which should only be opened immediately before use and sealed immediately afterwards.

5.4 Blanks

There are 3 types of blanks.

- a) Filter batch blank – for each new batch of filters to be used for sampling, prior to sampling, select 1 filter for every 25 filters for analysis as a normal sample for quality and background fibrous contamination. If the blank yields fibre counts greater than 5 fibres/100 graticule fields, the cause for contamination should be investigated or reject the entire batch of filters.
- b) Field blank – from a satisfactory batch of filters, select 1 filter for every 25 filters to be taken to the sampling area and then analysed as a normal sample, but with caps removed, replaced immediately and without having air drawn through it, or having been attached to the worker. If this “blank” yields fibre counts greater than 5 fibres/100 graticule fields, the entire sampling and analytical procedure should be examined carefully to find the cause of the contamination.
- c) Laboratory media blank – from a satisfactory batch of filters, select 1 filter for analysis as a normal sample if contamination due to laboratory sources is suspected.

A minimum of one field blank and one laboratory media blank should be analysed with each batch of samples.

When the field blank count exceeds 5 fibres/100 graticule fields, and also exceeds 10 % of the actual sample fibre count/100 graticule fields, the samples represented by the field blank are not considered acceptable for assessment of worker exposure. However, the determination can be permitted by regulations for indicating compliance with the exposure standard. For example, if the estimated exposure

is less than that permitted fibre concentration even with the contamination, this is a conservative estimate of compliance.

EXAMPLE The fibre count of blank filter was 7 fibres/100 graticule fields (i.e. 0,07 fibres/fields) while the sample yielded 20 fibres in 100 graticule fields. As the sample volume is 2 000 l of air, the fibre concentration is 0,005 fibre/cm³.

$$\frac{\text{Blank count}}{\text{Sample count}} = \frac{0,07}{0,20} \times 100\% = 35\% \tag{1}$$

As the percentage exceeds 10 % and the blank count exceeds 5 fibres/100 graticule fields, the sample is rejected for worker exposure assessment. The cause of contamination shall be found and corrected. However, for clearance testing, the sample is accepted as the estimated exposure of 0,005 fibre/cm³ complies with the clearance guideline of 0,01 fibre/ cm³.

For low sample counts as in the above example, blank counts must not be subtracted. It can be better to use filters with low blank fibre counts of less than 2,5 fibres/100 fields. For elevated sample counts as in worker exposure assessment, blank counts may be subtracted and be properly noted.

5.5 Sample duration and volume

5.5.1 Single sample

Taking into account the filter loading considerations detailed in 5.2, the duration *t*, in minutes, for each single sample may be determined from using Formula (2):

$$t = \frac{A}{a} \times \frac{E}{c_{\text{exp}}} \times \frac{1}{q} \tag{2}$$

where

- A* is the effective filter area, in square millimetres;
- a* is the graticule field, in square millimetres;
- c_{exp}* is the average fibre concentration, in fibre per cubic centimetre, expected to occur during the single sample duration;
- E* is the required filter loading, in fibres per graticule field;
- q* is the flowrate, in cubic centimetres per minute.

Table 1 — Single sample durations

Expected fibre concentration	t_{\min}^a	$t_{\text{recommended}}^b$	t_{\max}^c
0,1	3,3 h	Full shift	Full shift
0,5	40 min	3,0 h	8,0 h
1	20 min	1,5 h	4,0 h
2	10 min	45 min	2,0 h
5	d	20 min	1,0 h
10	d	10 min	30 min
20	d	10 min	10 min

a 0,4 fibres/graticule area is equivalent to 50 fibres/mm².
b 2 fibres/graticule field.
c 5 fibres/graticule field.
d Sampling periods shorter than 10 min are not recommended.

To provide guidance on the selection of single sample duration, [Table 1](#) lists recommended single sample durations based on 2 fibres/graticule field. If it is not possible to use these values, the minimum and maximum durations allow a choice to be made while still remaining within the constraints of [5.2](#). If the concentration is not known and the objective is compliance sampling, the single sampling duration should preferably be that recommended for the appropriate limit.

5.5.2 Clearance sample

The low expected fibre concentration requires adjusting the sample duration and sample volume. [Table 2](#) lists examples of limits of detection and quantitation are calculated from Formula 2.

Table 2 — Examples of Fibre Densities and Limit of Detection (LOD)

Fibre density on filter ^a		Fibre concentration in air, fibres/cm ³			
Fibres per 100 graticules	Fibres/mm ²	500 l air sample	1000 l air sample	2000 l air sample	3000 l air sample
200	255	0,20	0,10	0,05	0,033
100	127	0,10	0,05	0,025	0,017
80	102	0,08	0,04	0,020	0,013
50	64	0,05	0,025	0,012	0,008
25	32	0,025	0,012	0,006 3	0,004
20	25	0,025	0,010	0,005	0,003 3
10	12,7	0,010	0,005	0,002 5	0,001 7
8	10,2	0,008	0,004	0,002	0,001 3
5 (LOD)	6,4	0,005 4	0,002 5	0,001 2	0,000 8

a Assume 385 mm² effective filter area and graticule field = 0,007 85 mm² for relatively clean (few particulates aside from fibres) filters.

5.6 Sampling strategy and records

Exposure measurements of inorganic fibres such as asbestos are carried out to meet the following objectives:

- to assess exposure relative to a regulatory exposure standard or occupational exposure limit value, and to enable better control measures to be implemented;

b) to provide estimates of exposure for epidemiological studies.

Sampling is conducted so that the results are representative of the worker's exposure to fibres under typical working conditions for a full shift. This may require taking multiple samples to cover the entire shift.

A personal sample is taken within the worker's breathing zone.^{[7][21]} Usually the filter is fastened to the lapel of the worker's jacket with the cowl pointing downwards. The worker carries the pump on a belt or in a pocket.

5.6.1 Clearance sampling

"Aggressive sampling"^[14] is conducted in cleaned enclosure after asbestos removal. It requires using forced-air equipment or brushes to dislodge free fibres and slow-speed fans to keep fibres suspended during sampling. Static samplers with cowls pointing downwards are placed 1 to 2 m above the floor at locations away from corners of the room and obstruction to avoid unusual air circulation pattern. The number of samples per unit floor area is generally specified by regulations.^[14]

5.6.2 Records

All data necessary for the determination of the fibre concentration shall be recorded, as well as sampling details. For example:

- a) sampling strategy, conditions, and locations;
- b) sampling media, equipment, and calibration;
- c) name and contact information of each person responsible for the sampling;
- d) microscope identification, resolution check and graticule calibration;
- e) number of fields and fibres counted for each sample;
- f) calculation of fibre count, upper and lower 95 % confidence limits, and overall uncertainty;
- g) name and contact information of each person responsible for the analysis and reporting;
- h) relevant information and notes.

6 Evaluation

6.1 Sample preparation

6.1.1 Cleaning slides and equipment

Clean conditions shall be maintained at all times.

Clean slides with lens tissue or industrial paper tissue and lay them on a clean surface, e.g. lens tissue sheet.

NOTE Some types of lens-tissue can produce small fibres which can contaminate the preparation.

Wipe the scalpel and tweezers with lens tissue and place them on a clean surface, e.g. lens tissue. When mounting a series of filters, the mounting tools shall be wiped clean before dealing with each sample.

6.1.2 Cutting the filter sample

Place a wedge cut from the filter sample on a clean glass slide. All cutting of the filter should be done with a scalpel using a rolling action. Do not use scissors. It is recommended that the wedge should be

approximately one quarter of the filter. The entire filter may be mounted, although this procedure does not allow for re-analysis in the event of failure in the clearing and mounting procedure.

6.1.3 Mounting the sample

The acetone-triacetin method^[2] and the dimethyl formamide-Euparal method^[9] are given in [Annexes A](#) and [B](#).

6.2 Microscope adjustment principles

Microscope adjustments shall be a daily routine and recorded. Follow the manufacturer's instructions while observing the following guidelines.

- a) The image of the light source shall be in focus and centred on the condenser iris of the annular diaphragm for true Kohler illumination.
- b) The object for examination shall be in focus.
- c) The illuminator field diaphragm, or field iris, shall be in focus, centred on the sample and opened only to the point where the field of view is illuminated.
- d) The phase rings (annular diaphragm and phase shifting elements) shall be concentric.
- e) The eyepiece graticule shall be in focus.
- f) Additional information is provided in [Annex E](#).

6.3 Eyepiece graticule calibration

Each combination of eyepiece, objective and graticule shall be calibrated with a stage micrometer ([Annex C](#)). Should any of the three be changed, the combination shall be recalibrated. For some microscopes, calibrations will change for observers with different interpupillary distances.

6.4 Microscope/counter performance assessment

As mentioned in [4.3.3](#), a detection limit test slide is available which will assist in the regular assessment of microscope and counter performance. A practical detection limit corresponding to block 5 on the HSE/NPL test slide Mark II, shall be achieved,^[14] while only parts of block 6 may be visible and none of block 7 should be visible at the working magnification.

In the NIOSH 7400 method, blocks 6 and 7 of HSE/NPL test slide Mark II shall be invisible.^[17]

These slides are still widely used, but no longer commercially available and have been replaced with HSL/ULO Mark III slides. Any of the variants of these slides can be used provided the calibration certificate states that at least one of the blocks of lines is completely invisible. The microscope shall be adjusted so that the visibility of the blocks of lines matches the description on the accompanying calibration certificate. The microscope shall be re-adjusted until this is so, or otherwise not used.^[22]

Studies^{[8],[9],[23]} have shown that for counting Amosite fibres, the majority of errors are due to sizing. For chrysotile fibres, the majority of errors are missing fibres due to oversight and result in a negative bias. By examining standard relocatable test slides regularly, one should be able to identify these errors

and improve on the quality of fibre counts. For each examination, a score is calculated from the number of absolute discrepancies between the reported and verified fibres in each field as shown in Formula (3).

$$\text{Score} = \left[1 - \frac{\text{no. of discrepancies}}{\text{no. of verified fibres}} \right] \times 100 \quad (3)$$

A counter should achieve a score of 50 or higher before being allowed to carry out fibre counting.^[8] Records of scores achieved by the counters should be kept to ensure their performance.

NOTE The number of absolute discrepancies have been shown to be linearly related to the sum of counting errors.^[9] The positive discrepancies are mainly due to sizing extra fibres. The negative discrepancies are mainly due to oversight of fibres.

Exchange of standard relocatable test slides with experienced laboratories for comparison and participation in a national or international proficiency testing program will help to ensure that valid results are being generated.

6.5 Counting and sizing fibres

6.5.1 Low power scanning

Place the slide with the label on the right hand side onto the microscope stage. Scan the entire filter wedge area with a total magnification using the 10X objective.

The margin normally covered by the filter holder gasket shall be free of dust and fibres. The dust loading of all viewing fields should have similar appearances with respect to total dust loading. If the observed fields show marked differences in loading or gross aggregation of fibres or dust, the slide shall be rejected. Another filter wedge shall be mounted. If the problem persists, the filter shall be rejected. If uneven loading is not observed, proceed with the analysis and record such observation in the test report.

6.5.2 Graticule field selection

After a satisfactory low power scan, change the microscope objective to 40X phase and focus on the plane in which the dust resides. Ensure that the phase rings remain concentric. Fields for counting shall be chosen at random throughout the entire area of the filter wedge. Although most of the fibres and dust will be found on the upper surface of the filter, it will be necessary to focus below (e.g. up to 10 µm) and slightly above the surface. When counting and sizing, constant use of the fine focus is necessary because of the small depth of field of a 40X objective (i.e. 2 µm to 3 µm). Do not count fields that lie approximately within 3 mm of the filter edge or within 1 mm of the cutting line.

6.5.3 Laboratory working conditions

Care shall be taken to provide a comfortable environment for fibre counting. An ergonomically designed chair shall be used, with the microscope eyepiece situated at an appropriate height for viewing. External lighting should be set at a level similar to the illumination level in the microscope to reduce eye fatigue. In addition, counters should take breaks from the microscope every one or two hours to limit fatigue.

6.5.4 Counting criteria

a) Rejection of fields

If more than one-eighth of a graticule field is covered by an agglomerate of fibres and/or particles, the graticule field shall be rejected and another selected. Such occurrences shall be recorded. Do not report rejected graticule fields in the total number counted.

b) Number of fibres and/or fields to be evaluated

Record the number of the fields examined and the number of fibres. At least 100 fibres shall be counted with a minimum of 20 graticule fields evaluated.

- c) A countable fibre is defined as any object having a maximum diameter less than 3 µm, an overall length greater than 5 µm and a length to width ratio greater than 3:1. Those fibres that are close to 5 µm in length should be assessed by comparing them with the 5 µm bar outside the graticule field. If the fibre is attached (or apparently attached) to a non-fibrous particle, it should be assessed as if the particle did not exist; however, only the length of the visible part of the fibre is considered, not the part obscured by the particle (except where a fibre passing through a particle can be seen to be continuous). Suitable pictures meeting the criteria d) to f) are given in Reference.[14]
- d) A countable fibre with both ends within the graticule field shall count as one; a countable fibre with only one end within the area shall count as half. An agglomerate of fibres which at one or more points on its length appears to be undivided but which at other points appears to divide into separate strands is known as a split fibre. Any other agglomerate in which fibres touch or cross one another is known as a bundle.
- e) A split fibre is evaluated as a single countable fibre if it meets the definition in c), the diameter being measured across the largest undivided part and not the split part.
- f) Fibres in a bundle area are evaluated individually if they can be distinguished sufficiently to determine that they meet the definition in d). If no individual fibres meeting this definition can be distinguished, the bundle shall be evaluated as a countable fibre if it as a whole meets the definition.

6.6 Calculation of fibre concentration

6.6.1 Single values

The fibre concentration c , in fibres per cubic centimetre, for each single sample duration, is determined according to Formula (4):

$$c = \frac{A}{a} \times \frac{N}{n} \times \frac{1}{q} \times \frac{1}{t} \quad (4)$$

where

- A is the effective filter area, in square millimetres (see [Annex D](#));
- a is the graticule counting area, in square millimetres (see [Annex C](#));
- N is the total number of fibres counted;
- n is the number of graticule fields observed;
- q is the flowrate of air through filter, in cubic centimetres per minute;
- t is the single sample duration, in minutes.

6.6.2 Time-weighted average values

When several samples of different sampling durations are taken, calculate the time-weighted average concentration c_{TW} , in fibres per cubic centimetre, from the single values as shown in Formula (5).

$$c_{TW} = \frac{\sum_{j=1}^k c_j t_j}{\sum_{j=1}^k t_j} \quad (5)$$

where

- c_j is the single value of concentration, in fibres per cubic centimetre;
- t_j is the single sample duration, in minutes;
- k is the total number of samples.

If the single sample durations, t_j referred to above are of equal duration, Formula (5) can be simplified to Formula (6):

$$c_{TW} = \frac{\sum_{j=1}^k c_j}{k} \quad (6)$$

7 Sources of errors and uncertainty

7.1 General

Because of the nature of the membrane filter method, it is not possible to know the “true” airborne fibre concentration of a given dust cloud. For this reason, the accuracy of the method is generally assessed relative to a consensus mean N among either laboratories or counters within an individual lab. In this International Standard, a single fibre count (n) determines a measurement error expressed in terms of an expanded uncertainty [ISO/IEC Guide 98-3] interval which is expected to enclose the mean N with 95 % confidence. Because of the skewed distribution of counts, a peculiarity of the uncertainty intervals is their asymmetry about the measured value n , thus requiring two numbers for specification.

7.2 Uncertainty

Errors introduced into the estimation of airborne fibres comprise sampling and analytical errors, which are considered predominantly random in nature with negligible bias or systematic component. The application of standard procedures and a reproducible routine is the only way of controlling most of the many sources of error inherent in the membrane filter method. The following list describes some common sources of error.

7.2.1 Sampling variables

- Poisson deposition
- sampling flowrate
- sampling time

7.2.2 Analytical variables

- operator counting bias
- effective filter area

- graticule area
- micrometer calibration

7.2.3 Variation dependence on the mean number N of fibres

Wide experience in the sampling and counting of asbestos fibres [17], [24] has led to the realization that measurement variation is dominated by inter-reader variation together with (Poisson) variation in the number of fibres deposited into a given area to be counted.

Poisson distribution. As often only small samples of the fibres deposited on the filter are counted, errors arise in the estimation of the total number of fibres on the entire filter face. Theoretically, the Poisson distribution defines the variation in fibre counts resulting from viewing randomly selected counting fields on the filter. If an area containing a mean of N fibres is counted, and if a Poisson distribution were appropriate to the counting results, the coefficient of variation (CV) is equal to $1/N^{1/2}$. As shown in Table 3, the expected CV is 10 % for 100 fibres and 32 % for 10 fibres.

Inter-counter and non-counting variability. It has been shown experimentally that the actual CV is greater than these theoretical values mainly because of the subjective components of the microscope operator, [24] with CV given approximately by Formula (7):

$$CV = \sqrt{N + S_r^2 N^2} / N \quad (7)$$

S_r is the relative standard deviation in the limit that N is equal to 100 or greater. S_r was reported in Reference [24] equal to approximately 0,2 in characterizing the intra-laboratory (combined inter-and intra-counter) variability in a specific laboratory. NIOSH 7400 [17] further reports inter-lab studies indicating an inter-lab S_r equal to about 0,45 (see 7.3).

7.2.4 Expanded uncertainty

Formula (7) suggests that if n is the number of counts taken in a single reading, then the distribution of $n - N / \sqrt{N + S_r^2 N^2}$, though unknown, depends only weakly on N , since mean and variance are independent of N . In fact, from many readings by a number of counters, with N equal to the mean across the counters, confidence intervals were determined [24] as Formula (8):

$$-1,8 \leq (n - N) / \sqrt{N + S_r^2 N^2} \leq +2,6 \quad (95 \% \text{ confidence level}) \quad (8)$$

By solving the two quadratic equations at the two extreme limits in Formula (8) for N in terms of n , confidence limits enclosing the consensus mean N , given single measurements n are easily obtained:

$$LCL[n] \leq N < UCL[n] \quad (95 \% \text{ confidence level}) \quad (9)$$

where LCL and UCL are given by Formulae (10) and (11):

$$LCL[n] = \frac{2n + 2,6^2 - \sqrt{(2,6^2 + 2n)^2 - 4(1 - 2,6^2 S_r^2)n^2}}{2(1 - 2,6^2 S_r^2)} \quad (10)$$

$$UCL[n] = \frac{2n + 1,8^2 + \sqrt{(1,8^2 + 2n)^2 - 4(1 - 1,8^2 S_r^2)n^2}}{2(1 - 1,8^2 S_r^2)} \quad (11)$$

NOTE Formulae (10) and (11) are identical to equations given in NIOSH 7400 with the confidence level expanded from 90 % to 95 %.

From these equations, the confidence limits accounting for both subjective counter and Poisson components for various fibre counts are calculated (Table 3) by taking $S_r = 0,2$ from Reference [14]. The intra-counter variability may be greater if quality control is poor.

Table 3 — Intra-counter ($S_r = 0,2$) 95 % confidence interval bracketing the consensus mean for various numbers (n) of fibres in a single count calculated by Formulae (10) and (11)

Number (n) of fibres	Lower confidence limit (LCL)	Upper confidence limit (UCL)	Expanded uncertainty %
5	1,6	13	(-68,160)
7	2,6	16	(-63,129)
10	4,2	21	(-58,110)
20	10	37	(-50,85)
50	29	85	(-42,70)
100	62	163	(-38,63)
200	127	319	(-36,60)

NOTE Also shown is the confidence interval expressed as expanded uncertainty asymmetric around n and stated relative to n

7.2.5 Uncertainty budget excluding counting errors

Various factors aside from counting errors can introduce random variation in measured values of asbestos concentrations.[14] Such factors are identified in Table 4.

Table 4 — Example of an uncertainty budget for non-counting variable

Variable	Estimated uncertainty	Uncertainty squared
Sampling flowrate	0,03	0,000 8
Sampling time	0,02	0,000 4
Masterstage micrometer	0,01	0,000 1
Calibration of submaster	0,01	0,000 1
Calibration of graticule	0,02	0,000 4
Area of exposed filter	0,05	0,002 5
Sum of squares		0,004 3
Square root of sum of squares = Overall uncertainty		0,066
Expanded uncertainty (coverage factor $k = 2$)		0,13 or 13 %

The uncertainty in Table 4 contributes in quadrature to the expanded uncertainty. Therefore, as 13 % is small relative to the values in Table 3, it may be reasonable to conclude that the contribution of non-counting errors to the total uncertainties is not significant in comparison to the Poisson and subjective errors.

NOTE 1 Non-counting errors are negligible only if consistently controlled.

NOTE 2 Inhomogeneous deposition of dust on the filter leads to gross errors, the magnitude of which cannot be estimated. Counting 20 or more fields ensures that minor divergence from randomness does not bias the result.

7.3 Inter-laboratory variability

The inter-laboratory variability is caused primarily by the random Poisson and inter-laboratory subjective components. From several inter-laboratory studies, the NIOSH 7400 method takes the value

of the subjective component of variability, S_r , as 0,45. The lower and upper 95 % confidence limits shown in Table 5 are then calculated from Formulae (10) and (11). The ratios of the upper limits to the lower limits are about 3 times for the intra-laboratory 95 % confidence interval with S_r equal to 0,2. For the inter-laboratory confidence interval with $S_r = 0,45$, the ratios are over 10 times. This may imply that multi-laboratory results using the membrane method for determining airborne fibres are less meaningful than a single dedicated lab because of the wide gap of the values between the upper and lower confidence limits.

Studies [8], [9], [23] using relocatable slides prepared from the AIHA-PAT test filters have shown that counting bias is the major source of inter-laboratory variability. For counting Amosite fibres, the majority of errors are due to sizing. For chrysotile fibres, the majority of errors are missing fibres due to oversight. In a recent international study of 5 countries [8] and over 250 analysts, a gradual improvement of proficiency was observed after the first round of testing. In a second round, 95 % of the analysts were able to perform satisfactorily in counting Amosite fibres. Their mean fibre count to the verified fibre count ratio was $0,993 \pm 0,164$. For chrysotile fibres, only 53 % of the analysts performed satisfactorily and their mean fibre count to the verified fibre count was $0,879 \pm 0,185$. The uncertainty associated with this negative bias was not included here in the uncertainty analysis. It is essential that relocatable test slides should be included in the internal quality control program, slide exchange with other laboratories and proficiency testing program in order to minimize intra- and inter-laboratory uncertainty.

Table 5 — Comparison of 95 % confidence intervals calculated from Formulae (10) and (11), bracketing consensus means and considering

Number of fibres (n)	95 % confidence interval inter-laboratory variability $S_r = 0,45$		95 % confidence interval intra-laboratory variability $S_r = 0,2$	
	Lower	Upper	Lower	Upper
	20	8	115	10
50	22	247	29	85
100	45	537	62	163
200	91	1063	127	319

7.4 Lowering of intra- and inter-laboratory variance

It has been demonstrated that a large contribution to variance in the method is the ability of counters to detect and properly characterize fibres, even when using the same properly set-up microscope. This variance can be monitored through application of the appropriate quality assurance measures. The use of a gridded cover slip in the mounting procedure allows re-examination of same fields of the sample by experienced counters, preferably from within and from outside laboratory, to determine “consensus” or “verified” fibre counts without contribution from the variance of fibre distribution across the filter. [25] In this way, standard relocatable test slides are established. They are then used to identify and rectify the errors of the counters in order to maintain intra- and inter-counter variance to an acceptable level with $S_r = 0.2$ and a lower overall uncertainty. Thus it is possible for gridded cover slips to be used for every field sample preparation, but this is not likely in practice. If only a portion of the filter is prepared with a regular cover slip, a further portion can be prepared with a gridded cover slip where needed.

8 Test report

The test report should include the following information:

- a reference to this International Standard (i.e. ISO 8672:2014);
- the sample identification number;
- the start and end of the sampling period;

- d) the flowrate during the sampling period;
- e) the type of sample: personal or static sample;
- f) the description of the location where the sample was taken;
- g) the results;
- h) any deviations from the sampling and the analytical procedure;
- i) any other information relevant to the method; and
- j) name and signature of each person responsible to the test report.

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Annex A (informative)

Slide mounting: acetone-triacetin procedure

This annex describes a procedure to prepare slide by the acetone-triacetin mounting procedure.^[2]

A.1 Reagents and accessories

A.1.1 Acetone and triacetin, both of reagent grade.

A.1.2 Hot block acetone evaporator, available commercially.

A.1.3 Micropipette or hypodermic syringe, with a 22 gauge needle.

A.2 Procedure

A.2.1 Switch on “hot block” acetone evaporator. When the “hot block” is ready, place the wedge, dust side up, on a slide.

A.2.2 Insert the slide with wedge into the receiving slot at base of “hot block”. Immediately, inject about 250 µl of acetone into the vaporization chamber with a slow, steady pressure. The wedge should be clear in 3 s to 5 s.

A.2.3 Use a glass marking pen to mark on the underside of the slide the outline of the cleared filter wedge.

A.2.4 Use a hypodermic syringe with a 22-gauge needle or a 5 µl micropipette to place 3,0 to 3,5 µl triacetin on the wedge.

A.2.5 Gently lower cover slip onto the wedge at a slight angle to reduce bubble formation.

A.2.6 Label the identification of the right hand sight of the slide.

A.2.7 Heating the cleared filter to approximately 50°C for 15 min accelerates the clearing process and enables analysis to proceed almost immediately thereafter. Otherwise, it is necessary to delay counting for about 24 h until the filter wedge has dissolved under the action of the triacetin.

A.2.8 Seal the edges of the cover slip to the slide using nail polish if the slide is to be kept indefinitely.

A.2.9 Proceed counting once the nail polish is dry.

Carry out acetone mounting in a well-ventilated area such as in fume hood. On no occasion should it be conducted in the vicinity of an open flame.

NOTE 1 Too much triacetin (as indicated by excess liquid emerging from the edges of the wedge) can cause fibre migration in about 4 weeks. Insufficient triacetin will result in uneven clearing of the granularity left from the acetone vapour clearing. Furthermore, the refractive index of the mounted sample will not be suitable for optimum visibility for some fine fibres.

NOTE 2 The procedure for mounting the whole filter is given in the Appendix 1 of Reference [14].

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Annex B (informative)

Permanent slide preparation: dimethyl formamide - Euparal procedure

This annex describes a procedure to prepare permanent or relocatable slide by the dimethyl formamide/Euparal mounting procedure.^{[9],[11],[26]}

B.1 Dimethyl formamide (DMF) solution

Prepare a solution of 2 ml of de-ionized water, 1,4 ml of dimethyl formamide and 0,6 ml of acetic acid. Discard the solution after 1 week.

B.2 Template for positioning filter wedge on to the relocatable cover slip

As illustrated in [Figure B.1](#), draw a 1" × 3" rectangle representing the position of a microscope slide on a piece of paper.

In the middle of the rectangle, draw a wedge of approximately 1/4 of a 25 mm filter.

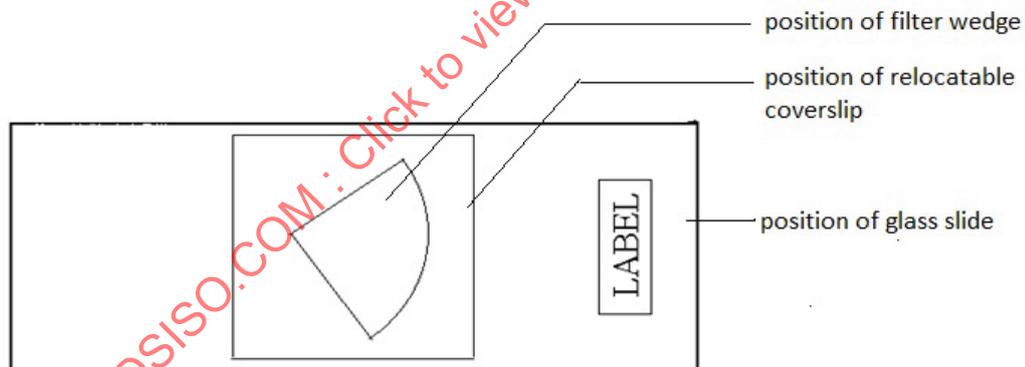


Figure B.1 — Example of a template for placing a filter wedge on a relocatable cover slip

B.3 Relocatable cover slip

Identify the coated side of the cover slip by scratching the corner with the tip of a tweezer. Examine the coated side of the cover slip at a total magnification of 400X to ensure the viewing fields are well defined and free of fibre-like particle contamination. Store the cover slip in a clean petri dish.

B.4 Cover slip

For regular sample, use non-gridded cover slip of No. 1-1/2, unless otherwise specified by microscope manufacturer.

B.5 Procedure

B.5.1 Place a clean microscope slide on the template.

B.5.2 Place a relocatable cover slip, coated side facing upward, on the microscope slide. Ensure the grids of relocatable fields are completely within the wedge of the template. The logo of cover slip has to be on the upper side of the grids of relocatable fields.

B.5.3 Use the template as a guide, place a wedge of filter sample, particle side facing down, on the the cover slip.

B.5.4 Ensure the grids of relocatable fields are completely within the wedge of filter sample.

B.5.5 Use a micropipette, add 20 μ l to 25 μ l of DMF solution in several driplets so that the wedge is completely wetted.

B.5.6 Place the assembly on a hotplate or drying oven at 60 °C for 30 min to clear the filter wedge or for a longer period until the smell of acetic acid is not detected any more.

B.5.7 Place the assembly back on the template. Add 1 drop of Euparal to the clear filter wedge.

B.5.8 Flip the cover slip upside down and slowly lower it at an angle on to the slide to ensure no bubbles trapped between the filter wedge and the slide. The logo of the cover slip should be below the grids of relocatable fields.

B.5.9 Place the assembly on a hotplate or drying oven at 60 °C for 60 min to set the Euparal. The slide is ready for examination. Under a microscope, the logo will appear on top of the relocatable fields.

B.5.10 As a standard relocatable test slide, the fibres enumerated in the selected fields should be verified by experienced analysts, preferably from another laboratory.

B.5.11 Records of the positions and number of fibres should be kept for future reference. An example of recording sheet of fibre counts of a relocatable slide is given in [Figure B.2](#).

B.5.12 Always store the slide flat and do not apply any pressure on the cover slip.

WARNING DMF is toxic, but has very low vapour pressure. Disposable gloves should be worn for handling the DMF solution. Drying of the slides should be done in a well-ventilated area to avoid inhalation of the vapour of the DMF solution.

DUST COUNTING RECORD (example only)

Counted by : _____
 Date : _____
 Microscope No. : _____
 Graticule type : Area mm² _____

Number of	
fibres	fields

O-bundles _____
 X-background not okay _____

EXAMPLE

	Name of Col.		Name of Col.								
row 1						row 1					
row 2						row 2					
row 3						row 3					
row 4						row 4					
row 5						row 5					
row 6						row 6					
row 7						row 7					
row 8						row 8					
row 9						row 9					
row 10						row 10					

$$c = \frac{\sum n_r}{\sum n_a} \times \frac{1}{V} \times F = \frac{\quad}{\quad} = \boxed{\quad} \text{ fibres/cm}^3$$

c is the concentration, in fibres per cubic centimeter
 $N = \sum n_r$ is the total number of fibres counted
 $n = \sum n_a$ is the effective filter area, in square millimetres
 a is the area of the counting field, in square millimetres
 V is the total flow, in cubic centimeters
 $F = \frac{A}{a}$ (constant factor)

Figure B.2 — Example of fibre count record of a relocatable slide

Annex C (normative)

Eyepiece graticule

C.1 Specifications of eyepiece graticule, ordering information and calibration

The graticule described in this method is the type G22 “Walton-Beckett” graticule.^[10] For each graticule, the desired diameter, d , of the circle to appear as $100 \pm 2 \mu\text{m}$ in the object plane, D , of the graticule and the overall diameter of the glass disc should both be specified in millimetres before ordering.

The following procedure is one of several methods for determining the diameter, d , of the circular counting area.

- a) Insert any available graticule into the eyepiece and focus so that the graticule grid is sharply in focus.
- b) Set the appropriate inter-pupillar distance, and, if applicable, reset the binocular head adjustment so that the “tube” length (and thus the magnification) remains constant.
- c) Ensure that the 40x phase objective is in place, and that the magnification changer position (if used) is known and recorded.
- d) Place a stage micrometer on the microscope object stage and focus the microscope onto the graduated lines.
- e) Measure the overall object length, l_o , of the graticule grid using the stage micrometer.
- f) Remove the graticule from the microscope and measure its actual overall grid length, l_a . This can be done by using a stage fitted with verniers.
- g) Calculate the diameter to be specified, d , using the following equation:

$$d = \frac{l_a}{l_o} D \quad (\text{C.1})$$

EXAMPLE

Step e) produced an object length of a Porton graticule of 108 μm .

Step f) produced an actual length of 4,50 mm.

Step g) produced a diameter of $(4,50/0,108) \times 0,1 = 4,17 \text{ mm}$.

It is also necessary to measure the overall diameter of the glass disc. In this case the disc diameter was found to be 17 mm. Thus a “Walton/Beckett” graticule of disc diameter 17 mm and circle diameter 4,17 mm should be specified for the above example.

C.2 Calibration of eyepiece graticules

Obtain a stage micrometer, preferably with a scale having 2 μm or 10 μm divisions and place it on the object stage of the microscope.

Make sure that the inter-pupillar distance of eyepieces is set correctly.

Note the objective magnification and any intermediate magnification used.