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

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*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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## 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 8672 was prepared by Technical Committee ISO/TC 146, *Air quality*, Sub-Committee SC 2, *Workplace atmospheres*.

Annexes A, B and C form an integral part of this International Standard.

Annexes D, E, F, G, H, J and K are for information only.

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## 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, may 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-dependance of the membrane filter method, it is important to apply this method with care and it shall be used in conjunction with a quality control scheme.

The World Health Organization has produced a variant of this method for use in Man-Made Mineral Fibres (MMMMF) industry workplaces<sup>[6]</sup>. A review of the whole field is given in<sup>[7]</sup>. It is recommended to use this review to assist in interpretation of the results of this method, particularly when applied outside the asbestos and MMMF manufacturing industries.

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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

### 1.1 General

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 4.3.4.

### 1.2 Limitations of the method

The method is applicable for routine sampling and sample evaluation necessary to assess personal exposure to fibres and to control their presence in occupational environments. This method can not 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 a full understanding of the workplace atmosphere. There are a variety of analytical methods which can be used to develop a full understanding of complex samples, e.g. polarizing light microscopy, electron microscopy.

With the parameters specified in this method, the theoretical lower detection limit for an 8 h-sample is  $0,02 \text{ fibres/cm}^3$ . However, the limit of practical use is often  $0,1 \text{ fibres/cm}^3$  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 artifacts 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.

The mounting medium proposed in this method has a refractive index of approximately 1,45. In workplace atmospheres where fibres with the refractive indices in the range of 1,4 to 1,5 may occur, the acetone-triacetone mounting method may not be appropriate and another mounting media shall be used.

## 2 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 filter is later transformed from an opaque membrane into a homogeneous optically transparent specimen. The fibres are then sized and counted using a phase contrast 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.

## 3 Sampling apparatus and technique

### 3.1 Filter

Membrane filters (mixed esters of cellulose or cellulose nitrate) of  $0,8 \mu\text{m}$  or less pore size and a diameter of 25 mm are preferred with printed grids.

### 3.2 Filter holder

It is necessary to use an open-faced filter holder fitted with a protective cowl. The distance between the cowl opening and the filter plane should be between one and half times and twice 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 than it. Figure 1 shows two possible arrangements.

The cowl helps to protect the filter from accidental contamination. A conducting cowl is preferred to a plastics 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 of larger pore size should be used.

The purpose of this supporting pad is to ensure an even distribution of air passing through the primary membrane.

3.3 Storage and transport

Fixatives shall not be used.

Experience has shown that fixing fibres to the filter surface with cytological or other types of fixatives is unnecessary and this shall not be done.

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

An alternative is to transfer the filter to a Petri dish in the following way.

In a dust-free area, using forceps, carefully remove each used filter from its holder, taking care to grasp

it on its unexposed edge. Place the filter, dust side up, in a plastics Petri dish or similar container. Fasten the filter to the bottom of the dish with one or two pieces of adhesive tape attached to the unexposed edge. After transportation, the filter can be removed easily from the dish with a surgical scalpel.

Pack the filter holders or Petri dishes into a rigid container with sufficient soft packing material to prevent both crushing and vibration of the filter. Samples shall be unambiguously labelled and caution is necessary to ensure that filters cannot be accidentally re-used. The filters should not be marked for this purpose because of the risk of damaging the filter.

3.4 Sampling pump

A portable battery-operated pump shall be used for personal sampling. The capacity of the battery shall be sufficient to operate continuously over the chosen sampling time. The flow shall be free from pulsation. As a minimum and tentative criterion, there shall be no visible vibration of a variable area flowmeter float when the flowmeter is connected to the filter holder.

Although some pumps are equipped with pulsation dampers, an external damper may have to be installed between the pump and the filter. Never run the pump without a filter.

Connecting tubing shall be constriction-proof and the connections shall be leakproof.

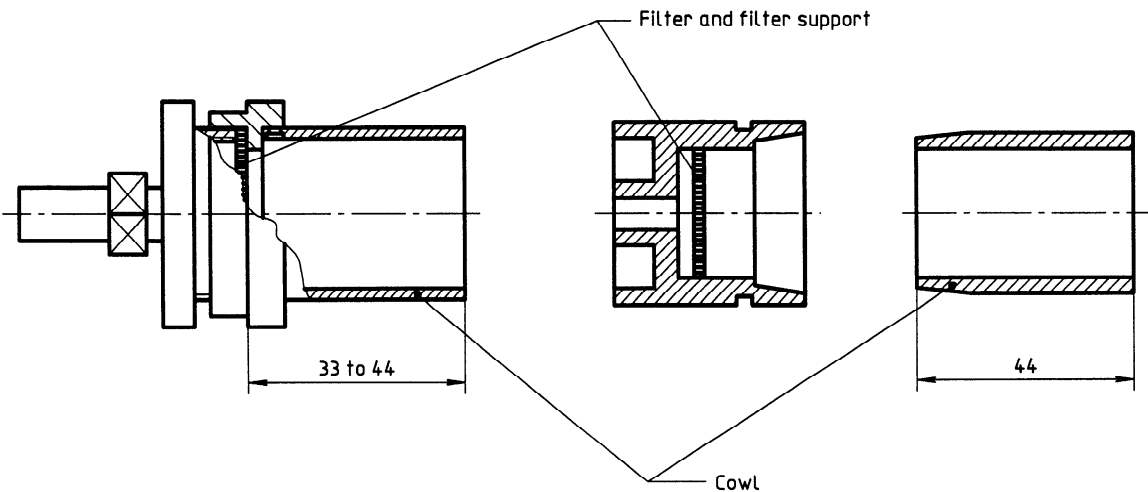


Figure 1 — Filter holders

### 3.5 Flowrate

The flowrate shall be adjusted to approximately 1 l/min, e.g. approximately 4 cm/s face velocity. The adjustment of sample density to the range specified in 3.6 should be done by adjusting sampling time as in 3.8. The flowrate shall be checked at least before and after sampling. If the difference from the initial flowrate is greater than 10 %, 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 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  $\pm 5$  % of the true flow (95 % confidence limit).

See annex E for flowrate calibration.

### 3.6 Acceptable fibre loadings on filters

#### 3.6.1 Minimum loading

The minimum filter loading should exceed 50 fibres/mm<sup>2</sup> (i.e. approximately 0,4 fibres/ Walton-Beckett graticule area). In special circumstances (e.g. when an indication of concentration with low precision is acceptable), it is permissible to lower the acceptable fibre loading to 20 fibres/mm<sup>2</sup> (i.e. approximately 0,15 fibres/Walton-Beckett graticule area).

The lowering of the acceptable fibre loading gives, at best, barely acceptable coefficients of variation. The limitations described in 1.2 should also be considered when measuring very low fibre concentrations.

#### 3.6.2 Maximum loading

The filter loading should not exceed a maximum of approximately 650 fibres/mm<sup>2</sup> (5 fibres/graticule area averaged for all counted fields) for the majority of sampling situations. This may need to be reduced to an average of about one fibre per graticule area when mixed dusts or agglomerates are present, and can sometimes be doubled when only fibres are present. Average filter loadings exceeding 5 fibres/graticule area tend to result in an underestimation and should be treated with caution.

### 3.7 Blanks

For each batch of filters used for sampling, and for every 25 filters in the batch, select one filter which has been subjected to the same treatment as normal samples, but without having the caps removed, having air drawn through it, or having been attached to the worker. If this "blank" yields fibre counts greater than 5 fibres/100 graticule areas, the entire sampling

and analytical procedure should be examined carefully to find the cause of the contamination.

When the blank count exceeds 5 fibres/100 graticule areas, and also exceeds 10 % of the actual sample fibre count/100 graticule areas, the samples represented by the blank are not considered acceptable for assessment of worker exposure.

However, the determination may still be useful for indicating compliance with the exposure standard. For example, if the estimated exposure is less than that permitted by regulations even with the contamination, this is a conservative estimate of compliance.

#### EXAMPLE

The fibre count of blank filter was 15 fibres/100 graticule areas (i.e. 0,15 fibres/area) while the sample yielded 108 fibres in 90 graticule areas (i.e. 1,20 fibres/area).

$$\frac{\text{Blank count}}{\text{Sample count}} (\%) =$$

$$\frac{0,15}{1,20} \times 100 = 12,5 \% \quad \dots (1)$$

As this percentage exceeds 10 %, the sample is rejected. Furthermore, because the blank count exceeded 5 fibres/100 graticule areas, the cause of contamination shall be found and corrected.

### 3.8 Recommended single sample duration

Taking into account the filter loading considerations detailed in 3.6, the duration  $t$ , in minutes, for each single sample may be determined from the following formula:

$$t = \frac{A}{a} \times \frac{L}{c_{\text{exp}}} \times \frac{1}{r} \quad \dots (2)$$

where

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

To provide guidance on the selection of single sample duration, table 1 lists recommended single sample durations based on 2 fibres/graticule area. If it is not possible to use these values, the minimum and maximum durations allow a choice to be made whilst still remaining within the constraints of 3.6. 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.

**Table 1 — Single sample durations**

Expected fibre concentration fibres/cm <sup>3</sup>	Single sample duration		
	$t_{\min}^{1)}$	$t_{\text{recommended}}^{2)}$	$t_{\max}^{3)}$
0,1	3,3 h	Full shift	Full shift
0,5	40 min	3 h	8 h
1	20 min	1,5 h	4 h
2	10 min	45 min	2 h
5	4)	20 min	1 h
10	4)	10 min	30 min
20	4)	10 min	10 min

1) 0,4 fibres/graticule area is equivalent to 50 fibres/mm<sup>2</sup>.  
 2) 2 fibres/graticule area.  
 3) 5 fibres/graticule area.  
 4) Sampling periods shorter than 10 min are not recommended.

Sampling time shall be measured within  $\pm 2,5\%$ .

NOTE 1 The timers or counters installed in some pumps are not always reliable.

### 3.9 Sampling strategy and records

Examples of strategy are given in annex D. All data necessary for the determination of the fibre concentration shall be recorded, as well as sampling details. For an example of a sampling record, see annex G.

## 4 Evaluation

### 4.1 Sample preparation

#### 4.1.1 Cleaning slides and equipment

Clean conditions shall be maintained at all times.

A dirty preparation area may result in sample contamination and erroneous results.

Clean slides with lens tissue or industrial paper tissue and lay them on a clean surface, e.g. lens tissue sheet. It is good practice to clean each coverslip with lens tissue immediately before use, to ensure that the surfaces are free from contamination.

**WARNING — Some types of lens-tissue can produce small fibres which may contaminate the preparation.**

Wipe the scalpel and forceps 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.

#### 4.1.2 Cutting the filter sample

Mounting of the total filter is preferable.

If it is necessary to cut the filter, all cutting should be done with a scalpel using a rolling action. Do not use scissors. It is recommended that the smallest piece mounted be wedge-shaped and approximately one-quarter or one-third of the filter.

#### 4.1.3 Mounting the sample

For mounting, use the acetone-triacetin method as described in annex A, unless a modified refractive index has to be used (see 1.2).

**WARNING — Acetone mounting shall be carried out only in a fume hood or fume cupboard. On no occasion should it be conducted in the vicinity of an open flame.**

### 4.2 Optical requirements

#### 4.2.1 Microscope equipment

Because microscopes with identical "specifications" can give quite different performances, it is necessary that the performance of proposed and existing microscopes be assessed by means of a detection limit test slide (see annex C). Provided this criterion is met, small departures from the recommended specifications in items d) and e) are permitted. It is also important that newcomers consult experienced workers before selecting microscopes for fibrous dust determination. The necessary specifications are as follows.

a) Light source, Köhler or Köhler type illumination.

It is preferable for the illuminator to be built-in but an external lamp with a plain mirror can be satisfactory. A variable light intensity control is necessary for both methods of illumination.

b) 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 focussing the condenser.

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

d) Objectives, a rotating nose-piece fitted with  $\times 10$  and  $\times 40$  parfocal phase-contrast achromatic objectives.



The  $\times 40$  objective shall have a numerical aperture (NA) of 0,65, achromatic. It shall have a phase ring of absorption not less than 65 % and not greater than 85 %.

- e) Binocular eyepieces chosen to give a total magnification of 400 to 600.

At least one eyepiece shall permit the insertion of a graticule. The compensating and focussing type are recommended. The use of body magnification changers is not recommended.

- f) Graticule (Walton-Beckett).

The diameter of the graticule in the object plane, when using the  $\times 40$  phase objective and an appropriate eyepiece shall be  $100\ \mu\text{m} \pm 2\ \mu\text{m}$ . See annex B for graticule specification, calibration, source of supply and ordering information.

- g) Accessories

Centering telescope or Bertrand Lens for checking that the phase rings in the condenser are centered with respect to those in the objective.

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

Stage micrometer which shall be subdivided into max. 10  $\mu\text{m}$  intervals.

Microscope slides which should be the best quality.

Coverslips of thickness (normally 0,17 mm) suitable for the microscope objective. Incorrect coverslip thickness will detract from the quality of the final image.

Hand operated counter or similar device.

#### 4.2.2 Microscope adjustment principles

Follow the manufacturer's instructions while observing the following guidelines.

- The image of the light source shall be in focus and centered on the condenser iris of the annular diaphragm for true Köhler illumination.
- The object for examination shall be in focus.
- The illuminator field iris shall be in focus, centered on the sample and opened only to the point where the field of view is illuminated.
- The phase rings (annular diaphragm and phase shifting elements) shall be concentric.

- e) The eyepiece graticule shall be in focus.

For more detailed information see annex H.

Microscope adjustments shall be a daily routine.

#### 4.2.3 Eyepiece graticule calibration

Each combination of eyepiece, objective and graticule shall be calibrated with a stage micrometer. Should any of the three be changed, the combination shall be recalibrated. For some microscopes, calibrations will change for observers with different interpupillary distances (see annex B for eyepiece graticule calibration procedures).

#### 4.2.4 Microscope/observer performance assessment

It is necessary that laboratories following this method should maintain contact with those having experience with it. As mentioned in 4.2.1, a detection limit test slide is available which will assist in the regular assessment of microscope and observer performance. A practical detection limit corresponding to block 5 on the HSE/NPL test slide Mark II, shall be achieved (see annex C for method of use and supplier). Exchange of microscope slides with experienced laboratories for comparison will help to ensure that valid results are being generated.

### 4.3 Counting and sizing fibres

#### 4.3.1 Low power scanning

Scan the entire filter area with a total magnification of  $\times 100$  to  $\times 150$  (i.e.  $\times 10$  objective).

The margin normally covered by the filter holder gasket shall be free of dust and fibres. 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 filter shall be rejected.

#### 4.3.2 Graticule field selection

After a satisfactory low power scan, change the microscope objective to  $\times 40$  phase and focus on the dust plane.

Ensure that the phase rings remain concentric. 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  $\mu\text{m}$ ) 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  $\times 40$  objective (i.e. 2  $\mu\text{m}$  to 3  $\mu\text{m}$ ). Fields for counting shall be chosen at random throughout the entire area of the filter or filter segments. If the grid of a filter obstructs the view, move the stage to an-

other field. Do not count fields that lie within 3 mm of the filter edge or within 2 mm of the cutting line, if any.

#### 4.3.3 Laboratory working conditions

The working practices and the working environment in a laboratory may influence systematically the level of reliability of the actual counting. This shall be controlled by a quality assurance scheme.

Some differences may appear when inter-laboratory comparisons are made which are due merely to different laboratory lighting conditions, different seating and computing arrangements, etc. Different ways of recording data may also cause some disagreement between the counters, due to the rate of visual fatigue.

The detailed writing of data involves the re-focussing of the eyes after viewing each field, whereas continuous registering with electrical or mechanical counters involves only a single period of continuous concentration.

#### 4.3.4 Counting criteria

##### a) Choice of fields

Graticule areas for counting shall be chosen at random so that they are representative of the whole exposed area of the filter and do not overlap.

One method is to traverse the filter on randomly chosen chords taking fields at random.

##### b) Rejection of fields

Graticule areas which include grid lines shall be rejected. If more than one-eighth of a graticule area is covered by an agglomerate of fibres and/or particles, the graticule area shall be rejected and another selected. Such occurrences shall be recorded.

##### c) Number of fibres and/or fields to be evaluated

At least 100 fibres shall be counted with a minimum of 20 graticule areas evaluated. It is not necessary to evaluate more than 100 graticule areas.

##### d) 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 : diameter ratio greater than 3:1, and which does not appear to touch any particle with a maximum diameter greater than 3 µm. Suitable pictures meeting the criteria d) to g) are given in [2].

##### e) A countable fibre with both ends within the graticule area 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.

##### f) A split fibre is evaluated as a single countable fibre if it meets the definition in d), the diameter being measured across the largest undivided part and not the split part.

##### g) 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.

### 4.4 Calculation of fibre concentration

#### 4.4.1 Single values

The fibre concentration  $c$ , in fibres per cubic centimetre, for each single sample duration is determined according to the following formula:

$$c = \frac{A}{a} \times \frac{N}{n} \times \frac{1}{r} \times \frac{1}{t} \quad \dots (3)$$

where

$A$  is the effective filter area, in square millimetres (see annex F);

$a$  is the graticule counting area, in square millimetres (see annex B);

$N$  is the total number of fibres counted;

$n$  is the number of graticule areas observed;

$r$  is the flowrate of air through filter, in cubic centimetres per minute;

$t$  is the single sample duration, in minutes.

An example of a counting record is given in annex J.

#### 4.4.2 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 follows:

$$c_{TW} = \frac{\sum c_i \times t_i}{\sum t_i} = \frac{c_1 \times t_1 + c_2 \times t_2 + \dots + c_n \times t_n}{t_1 + t_2 + \dots + t_n} \quad \dots (4)$$

where

- $c_i$  is the single value of concentration, in fibres per cubic centimetre;
- $t_i$  is the single sample duration, in minutes;
- $n$  is the total number of samples.

If the single sample durations,  $t_i$ , referred to above are of equal duration, equation (4) can be simplified as follows:

$$c_{TW} = \frac{\sum c_i}{n} = \frac{c_1 + c_2 + \dots + c_n}{n} \quad \dots (5)$$

## 5 Sources of errors

### 5.1 General

Errors introduced into the estimation of airborne fibres comprise sampling and analytical errors, each of which has a systematic and random 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.

### 5.2 Systematic errors

#### 5.2.1 Sampling

- Flowrate.
- Sampling time.
- Non-representative or biased sampling.
- Contamination, deliberate or accidental.

#### 5.2.2 Analytical

- Effective filter area.
- Counting area.
- Counting criteria.
- Filter mounting.
- Counting operator bias.
- Microscope.
- Contamination.

### 5.3 Random errors

#### 5.3.1 Sampling

- Flowrate variability.
- Random fluctuations of the airborne dust cloud.

#### 5.3.2 Analytical

- Counting operator variability.
- Fibre distribution on the filter. Non-random deposition of dust on the filter leads to gross errors, the magnitude of which cannot be estimated. Twenty or more fields shall be counted to ensure that minor divergence from randomness does not bias the result.
- Poisson distribution. As 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 a minimum of 100 fibres is counted, and if a Poisson distribution were appropriate to the counting results, the relative standard deviation of the fibre counts would be  $\pm 10\%$ . It has been shown experimentally that the actual distribution of fibre counts can depart from that of Poisson, in which case the standard deviation may be greater.

### 5.4 Overall accuracy

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 it is not possible to assess the likely accuracy of the method. Even the precision (or repeatability) of the method is difficult to quantify because of systematic inter- and intra-laboratory errors which tend to arise. By "randomly" selecting observers and laboratories, these systematic errors take on a random nature so that it may be possible to provide estimates of empirical precision (i.e. the closest possible approach to a statement of accuracy for a method with no known "true" values).

Much work has been done in an attempt to arrive at these estimates, and until now only partial conclusions have been reached. One of these conclusions is that the theoretical Poisson distribution (see 5.3.2) contributes a  $\pm 95\%$  confidence interval of  $\pm 20\%$  for a total of 100 fibres counted, or up to about  $\pm 35\%$  for only 40 fibres counted in 100 graticule areas.

Other sources of random and systematic errors add significantly to the uncertainty in estimating the airborne fibre concentration.

## 5.5 Presentation of results

At present there is insufficient information available to determine at what level the reliability of the method becomes so poor that the results have little meaning. It is clear that this will not be a single value, but will be a range depending upon at least the absolute fibre concentration and the concentration relative to other dust. There appears to be general agreement amongst those experienced in the field, that these limits lie somewhere in the range of 0,1 fibres/cm<sup>3</sup> to 0,5 fibres/cm<sup>3</sup>, depending on a variety of conditions. In view of this situation, and the inherent variability of the method, all calculated values of less than 0,1 fibres/cm<sup>3</sup> should usually be reported as "less than 0,1 fibres/cm<sup>3</sup>". All higher values should be rounded off to the first decimal place, and to two significant figures.

## 6 Test report

The test report should include the following information:

- a) a reference to this International Standard;
- b) the sample identification number;
- c) 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.

An example of a sampling record is given in annex G.

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