
**Ambient air — Determination of
asbestos fibres — Indirect-transfer
transmission electron microscopy
method**

*Air ambiant — Dosage des fibres d'amiante — Méthode par
microscopie électronique à transmission par transfert indirect*





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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

This second edition cancels and replaces the first edition (ISO 13794:1999), which has been technically revised. The main changes compared to the previous edition are as follows:

- the use of electronic display systems with measurement software is permitted;
- the maximum particulate loading for TEM specimens is increased from 10 % to 25 %;
- a simplified fibre identification procedure for investigation of known sources of the regulated asbestos varieties and richterite/winchite asbestos is permitted;
- the reporting requirements have been changed to permit reporting of the concentrations of fibres and bundles longer than 5 µm and/or the concentrations of PCM equivalent fibres without the requirement to report the concentrations of structures equal to or greater than 0,5 µm;
- there is no requirement to report the 95 % confidence intervals of the fibre concentrations.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document is applicable to the measurement of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation of any atmosphere. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre size and type are the relevant parameters for evaluation of the inhalation hazards, a fibre counting and measuring technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos, and therefore there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This document is based on transmission electron microscopy, which has adequate resolution to allow for the detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis becomes prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective and instrumental factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of asbestos fibres is required.

In addition to single fibres and bundles, asbestos is often found in air samples as very complex, aggregated structures, which may or may not be also aggregated with other particles. The number of asbestos fibres and bundles incorporated in these complex structures often exceeds the number of isolated fibres and bundles observed, and many of them may be completely obscured in direct-transfer transmission electron microscope (TEM) preparations. The method defined in this document incorporates specimen preparation procedures that result in the selective concentration of asbestos fibres and the removal of organic, water-soluble and acid-soluble materials. These procedures have the effect of dispersing the majority of the complex clusters and aggregates of fibres into their component fibres and bundles so that the asbestos in the air sample can be more accurately quantified. All of the feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion on to a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. Although this method results in dispersal of complex clusters and aggregates, it minimizes other effects on the size distribution of fibres and fibre bundles.

This document requires a very detailed and logical procedure is used to reduce the subjective aspects of the measurement. The method of data recording specified in the document is designed to allow re-evaluation of the fibre counting data as new medical evidence becomes available.

This document describes the method of analysis for a single air filter. However, one of the largest potential errors in characterizing asbestos in ambient atmospheres is associated with the variability between filter samples. For this reason, it is necessary to design a replicate sampling scheme in order to determine the standard's accuracy and precision.

Comparison of results obtained using this indirect-transfer procedure with those from the direct-transfer procedure cannot be done a priori. This can only be achieved by a site-specific inter-comparison study that takes into account the fibre size and type of asbestos, and also the nature of the source of the airborne asbestos.

Ambient air — Determination of asbestos fibres — Indirect-transfer transmission electron microscopy method

1 Scope

This document specifies a reference method using transmission electron microscopy for the determination of airborne asbestos fibres and structures in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation for asbestos structures in any atmosphere. The specimen preparation procedure incorporates ashing and dispersion of the collected particulate, so that all asbestos is measured, including the asbestos originally incorporated in particle aggregates or particles of composite materials. The lengths, widths and aspect ratios of the asbestos fibres and bundles are measured, and these, together with the density of the type of asbestos, also allow the total mass concentration of airborne asbestos to be calculated. The method allows determination of the type(s) of asbestos fibres present. The method cannot discriminate between individual fibres of the asbestos and elongate fragments (cleavage fragments and acicular particles) from non-asbestos analogues of the same amphibole mineral^[12].

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 4225, *Air quality — General aspects — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 4225 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

acicular

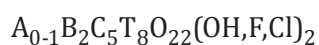
shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like

[SOURCE: ISO 10312:1995, 3.1]

3.2

amphibole

group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:



where

A is K, Na;

B is Fe^{2+} , Mn, Mg, Ca, Na;

C is Al, Cr, Ti, Fe^{3+} , Mg, Fe^{2+} ;

T is Si, Al, Cr, Fe^{3+} , Ti.

Note 1 to entry: In some varieties of amphibole, these elements can be partially substituted by Li, Pb, or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124° .

[SOURCE: ISO 10312:1995, 3.2]

3.3

amphibole asbestos

amphibole in an asbestiform habit

[SOURCE: ISO 10312:1995, 3.3]

3.4

analytical filter

filter through which an aqueous dispersion of ash from the sample collection filter is passed, and from which transmission electron microscope specimen grids are prepared

[SOURCE: ISO 13794:1999, 2.4]

3.5

analytical sensitivity

calculated airborne asbestos structure concentration in structures/litre, equivalent to counting of one asbestos structure in the analysis

Note 1 to entry: It is expressed in structures/litre.

Note 2 to entry: This method does not specify a unique analytical sensitivity. The analytical sensitivity is determined by the needs of the measurement and the conditions found on the prepared sample.

[SOURCE: ISO 10312:1995, 3.4]

3.6

asbestiform

specific type of mineral fibrosity in which the fibres and fibrils possess high tensile strength and flexibility

[SOURCE: ISO 10312:1995, 3.5]

3.7

asbestos

group of silicate minerals belonging to the serpentine and amphibole groups which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong fibres when crushed or processed

Note 1 to entry: The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), crocidolite (12001-28-4), grunerite asbestos (Amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4). Other varieties of asbestiform amphibole, such as richterite asbestos and winchite asbestos^[18], are also found in some products such as vermiculite and talc.

[SOURCE: ISO 10312:1995, 3.6]

3.8**asbestos structure**

individual fibre, or any connected or overlapping grouping of asbestos fibres or bundles, with or without other particles

[SOURCE: ISO 10312:1995, 3.7]

3.9**ashed filter blank**

fibre count made on transmission electron microscope specimens prepared by the indirect procedure from a blank membrane filter of the type used for collection of air samples

[SOURCE: ISO 13794:1999, 2.9]

3.10**aspect ratio**

ratio of length to width of a particle

[SOURCE: ISO 10312:1995, 3.8]

3.11**blank**

structure count made on transmission electron microscope specimens prepared from an unused filter, to determine the background measurement

[SOURCE: ISO 10312:1995, 3.9]

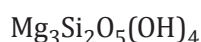
3.12**camera length**

equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action

[SOURCE: ISO 10312:1995, 3.10]

3.13**chrysotile**

fibrous mineral of the serpentine group, which has the nominal composition:



Note 1 to entry: Most natural chrysotile deviates little from this nominal composition. In some varieties of chrysotile, minor substitution of silicon by Al^{3+} may occur. Minor substitution of magnesium by Al^{3+} , Fe^{2+} , Fe^{3+} , Ni^{2+} , Mn^{2+} and Co^{2+} may also be present. Chrysotile is the most prevalent type of asbestos.

[SOURCE: ISO 10312:1995, 3.11]

3.14**cleavage**

breaking of a mineral along one of its crystallographic directions

[SOURCE: ISO 10312:1995, 3.12]

3.15**cleavage fragment**

fragment of a crystal that is bounded by cleavage faces

Note 1 to entry: Crushing of non-asbestiform amphibole generally yields elongated fragments that conform to the definition of a fibre, but rarely have aspect ratios exceeding 30:1.

[SOURCE: ISO 10312:1995, 3.13, modified — Note 1 to entry added.]

3.16

cluster

structure in which two or more fibres, or fibre bundles, are randomly oriented in a connected grouping

[SOURCE: ISO 10312:1995, 3.14]

3.17

direct-transfer blank

structure count made on transmission electron microscope specimens prepared by the direct-transfer procedure from a blank filter of the type used for filtration of aqueous dispersions of ash

[SOURCE: ISO 13794:1999, 2.17]

3.18

d-spacing

distance between identical adjacent and parallel planes of atoms in a crystal

[SOURCE: ISO 10312:1995, 3.15]

3.19

electron diffraction

technique in electron microscopy by which the crystal structure of a specimen is examined

[SOURCE: ISO 10312:1995, 3.16]

3.20

electron scattering power

extent to which a thin layer of substance scatters electrons from their original directions

[SOURCE: ISO 10312:1995, 3.17]

3.21

empty beaker blank

fibre count made on transmission electron microscope specimens prepared by the indirect procedure using an empty beaker as the initial sample

[SOURCE: ISO 13794:1999, 2.21]

3.22

energy dispersive X-ray analysis

measurement of the energies and intensities of X-rays by use of a solid state detector and multi-channel analyser system

[SOURCE: ISO 10312:1995, 3.18]

3.23

eucentric

condition when the area of interest of an object is placed on a tilting axis at the intersection of the electron beam with that axis and is in the plane of focus

[SOURCE: ISO 10312:1995, 3.19]

3.24

field blank

filter cassette that has been taken to the sampling site, opened and then closed and which is used to determine the background structure count for the measurement

[SOURCE: ISO 10312:1995, 3.20]

3.25**fibril**

single fibre of asbestos, which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances

[SOURCE: ISO 10312:1995, 3.21]

3.26**fibre**

elongated particle which has parallel or stepped sides

Note 1 to entry: For the purposes of this document, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.

[SOURCE: ISO 10312:1995, 3.22]

3.27**fibre bundle**

structure composed of parallel, smaller diameter fibres attached along their lengths

Note 1 to entry: A fibre bundle may exhibit diverging fibres at one or both ends.

[SOURCE: ISO 10312:1995, 3.23]

3.28**fibrous structure**

fibre, or connected grouping of fibres, with or without other particles

[SOURCE: ISO 10312:1995, 3.24]

3.29**funnel blank**

structure count made on transmission electron microscope specimens prepared by the direct-transfer method from a filter used for filtration of a sample of distilled water

[SOURCE: ISO 13794:1999, 2.29]

3.30**habit**

characteristic crystal growth form or combination of these forms of a mineral, including characteristic irregularities

[SOURCE: ISO 10312:1995, 3.25]

3.31**limit of detection**

calculated airborne fibre concentration in structures/l, equivalent to the upper 95 % confidence limit of 2,99 structures predicted by the Poisson distribution for a count of zero structures

[SOURCE: ISO 10312:1995, 3.26]

3.32**matrix**

structure in which one or more fibres, or fibre bundles, touch, are attached to, or partially concealed by, a single particle or connected group of non-fibrous particles

[SOURCE: ISO 10312:1995, 3.27]

3.33

Miller index

set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes

[SOURCE: ISO 10312:1995, 3.28]

3.34

PCM equivalent fibre

fibre of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

[SOURCE: ISO 10312:1995, 3.29]

3.35

PCM equivalent structure

fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

[SOURCE: ISO 10312:1995, 3.30]

3.36

pixel

smallest image-forming element to which a grey level is assigned

[SOURCE: ISO 23900-6:2015, 2.10]

3.37

primary structure

fibrous structure that is a separate entity in the transmission electron microscope image

[SOURCE: ISO 10312:1995, 3.31]

3.38

replication

procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made

[SOURCE: ISO 10312:1995, 3.32]

3.39

selected area electron diffraction

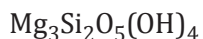
technique in electron microscopy in which the crystal structure of a small area of a sample is examined

[SOURCE: ISO 10312:1995, 3.33]

3.40

serpentine

group of common rock-forming minerals having the nominal formula:



[SOURCE: ISO 10312:1995, 3.34]

3.41

structure

single fibre, fibre bundle, cluster or matrix

[SOURCE: ISO 10312:1995, 3.35]

3.42**twinning**

occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law

[SOURCE: ISO 10312:1995, 3.36]

3.43**unopened fibre**

large diameter asbestos fibre bundle that has not been separated into its constituent fibrils or fibres

[SOURCE: ISO 10312:1995, 3.37]

3.44**zone-axis**

line or crystallographic direction through the centre of a crystal that is parallel to the intersection edges of the crystal faces defining the crystal zone

[SOURCE: ISO 10312:1995, 3.38]

4 Symbols and abbreviated terms

eV	electron volt
kV	kilovolt
l/min	litres per minute
µg	microgram (10^{-6} grams)
µm	micrometre (10^{-6} metre)
nm	nanometre (10^{-9} metre)
W	Watt
DMF	Dimethylformamide
ED	Electron diffraction
EDXA	Energy dispersive X-ray analysis
FWHM	Full width, half maximum
HEPA	High efficiency particle absolute
MEC	Mixed esters of cellulose
PC	Polycarbonate
PCM	Phase contrast optical microscopy
SAED	Selected area electron diffraction
SEM	Scanning electron microscope
STEM	Scanning transmission electron microscope
TEM	Transmission electron microscope
UICC	Union Internationale Contre le Cancer

5 Type of sample

The method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn.

6 Range

The upper limit for the range of concentration that can be measured on the analytical filter is 7 000 structures/mm². The lower limit of the range that can be measured on the analytical filter corresponds to the detection of 2,99 structures in the area of specimen examined. Measurement of concentrations lower than 1 structure/mm² can be achieved. The air concentrations represented by these values are a function of the volume of air sampled and the degree of dilution or concentration selected during the specimen preparation procedures. The method is particularly applicable to measurements in areas with high suspended particulate concentrations (exceeding 25 µg/m³), or where detection and identification of asbestos fibres are likely to be prevented or hindered by other types of particulate in direct-transfer TEM preparations. In theory, there is no lower limit to the dimensions of asbestos fibres that can be detected. In practice, microscopists vary in their ability to detect very short asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

The method also includes provision for measurement of the concentrations of fibres with sizes thought to be of particular biological importance (fibres and bundles >5 µm), and also fibres of sizes defined in regulations (PCM equivalent fibres).

7 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air, concentrating the sample during specimen preparation, and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of an examined TEM specimen is controlled by the total suspended particulate concentration remaining after the ashing and aqueous dispersal steps, and this depends on the chemical nature of the suspended particulate. For total suspended particulate concentrations of approximately 10 µg/m³, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 l of air, an analytical sensitivity of 0,5 structure/l can be obtained, equivalent to a limit of detection of 1,8 structure/l, if an area of 0,195 mm² of the TEM specimens is examined. For fibres longer than 5 µm, examined at lower magnifications, this limit of detection can be reduced by a further order of magnitude. Lower limits of detection can be achieved by increasing the area of the TEM specimens that is examined, or by concentration of the sample during specimen preparation. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre.

8 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a capillary-pore polycarbonate (PC) membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,8 µm by means of a battery-powered or mains-powered pump. A portion of the filter is ashed in an oxygen plasma^[22], and the residual ash is dispersed in distilled water with adjustment of the pH to between 3,0 and 4,0 using acetic acid. Analytical filters are then prepared by filtration of known volumes of this aqueous dispersion through either capillary-pore polycarbonate membrane filters of maximum pore size 0,2 µm or cellulose ester membrane filters of maximum pore size 0,22 µm. TEM specimens are prepared from polycarbonate analytical filters by a carbon replication procedure^[10] in which a thin film of carbon is applied to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film carbon replica which bridges the openings in the TEM specimen grid, and which supports each particle from the analytical filter.

original position. Cellulose ester analytical filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma^[22] to ensure that all particles are exposed^[11]. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative fibre count on randomly-selected grid openings. If the selected TEM specimen grid has too high a particulate or fibre loading, another specimen grid with a lower filtered aliquot shall be selected for analysis. In the TEM analysis, electron diffraction (ED)^[7] is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA)^[6]. For a number of reasons, it is not possible to identify each fibre unequivocally, and fibres are classified according to the techniques which have been used to identify them. A simple code is used to record, for each fibre, the manner in which it was classified. The fibre classification procedure is based on successive inspection of the morphology, the selected area electron diffraction pattern, and the qualitative and quantitative energy dispersive X-ray analyses. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone-axis ED.

In addition to isolated fibres, ambient air samples often contain more complex aggregates of fibres, with or without other particles. Some particles are composites of asbestos fibres with other materials. Individual fibres and these more complex structures are referred to as “asbestos structures”. The indirect specimen preparation procedure permits the majority of these complex structures to be dispersed into their constituent fibres and fibre bundles, allowing more precise quantification than is possible using the direct-transfer procedure. A coding system is used to record the type of fibrous structure, and also to provide the optimum morphological description of each structure. The two codes remove from the microscopist the requirement to interpret the fibre counting data and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibres. The procedure permits a minimum required fibre identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fibre, and the degree of success is recorded for each fibre. The lengths and widths of all classified structures are recorded. The number of asbestos structures found on a known area of the microscope sample, together with the volume of air filtered through the sample collection filter, the proportion of the sample collection filter that was ashed, the proportion of the aqueous dispersion that was filtered, and the area of the analytical filter are used to calculate the airborne concentration of asbestos in asbestos structures/litre of air. The mass concentration of asbestos is calculated using an assumed density for the asbestos variety, and the widths and lengths of the fibres.

9 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water (9.1).

9.1 Water, fibre-free.

A supply of freshly distilled, fibre-free water, or another source of fibre-free, pyrogen-free water shall be used. Freshly-distilled water, filtered through a 0,1 µm pore size MEC filter, has been found satisfactory.

9.2 Chloroform, analytical grade, distilled in glass, preserved with a volume fraction of 1 % ethanol.

9.3 1-Methyl-2-pyrrolidone.

9.4 1,2-Diaminoethane (Ethylene diamine).

9.5 Dimethylformamide.

9.6 Glacial acetic acid.

9.7 Acetone.

10 Apparatus

10.1 Air sampling

10.1.1 Filter cassette

Field monitors, comprising 25 mm to 50 mm diameter conductive three-piece cassettes shall be used for sample collection. The cassette shall be loaded with either a capillary pore polycarbonate filter of maximum pore size 0,4 µm or a cellulose ester (MEC or cellulose nitrate) filter of maximum pore size 0,8 µm. If push-fit cassettes are used, when the filter is in position, a shrink cellulose band or adhesive tape shall be applied to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

Representative filters from the filter lot shall be analysed as described in [12.8](#) for the presence of asbestos structures, and also tested for suitability as described in [Annex H](#), before any are used for air sample collection.

NOTE This method permits the use of larger pore size cellulose ester filters for sample collection than the maximum pore size permitted in the direct-transfer method ISO 10312^[2]. The smaller maximum pore size is specified in ISO 10312 in order to ensure that collected particulate and fibres are retained close to the surface of the filter, which is required if the particulate and fibres are to be transferred to the TEM grid with high efficiency by the direct-transfer procedure. In this indirect-transfer method, the depth of penetration of particulate and fibres into the filter medium during sample collection is unimportant, provided that they do not pass through the filter.

10.1.2 Sampling pump

The sampling pump shall be capable of a flow-rate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4,0 cm/s and 87,0 cm/s. The sampling pump used shall provide a non-fluctuating airflow through the filter and shall maintain the initial volume flow-rate to within ±10 % throughout the sampling period. A constant flow or critical orifice-controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flow-rate of each pump is also required.

NOTE The sampling efficiency for a particular particle size varies with the face velocity. Depending on the size distribution of the airborne particles, the analytical result can vary with face velocity.

10.1.3 Stand

A stand shall be used to hold the filter cassette at the desired height for sampling and shall be isolated from the vibrations of the pump ([10.1.2](#)).

10.1.4 Personal sampling

For collection of air samples intended to represent the exposure of an individual, the filter cassette shall be attached within the breathing zone of the individual, i.e. within 25 cm of the worker's nose and mouth. Air sampling filter cassettes may be attached to a collar or lapel, with the open end of the cassette facing downwards.

10.1.5 Flowmeter

A calibrated flowmeter with an appropriate range and accurate to within 2,5 % of the indicated flow rate is required for calibration of the air sampling system.

Ensure that the flowmeter is clean before use in order to avoid transfer of asbestos contamination from the flowmeter to the sample being collected.

10.2 Specimen preparation laboratory

Asbestos, particularly chrysotile, may be present in at trace levels in some laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibres which may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that, during preparation, contamination of TEM specimens by any extraneous asbestos fibres is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the requirements specified in 12.8. Preparation areas with filtered air are recommended. However, it has been established that work practices in specimen preparation appear to be more important than the type of clean handling facilities in use. Use of a dedicated TEM preparation area and isolation of this area from other activities such as bulk asbestos analysis is a practical way of avoiding cross contamination. Transfer of extraneous asbestos into the TEM preparation area can be avoided by the use of dedicated laboratory clothing and cleaning of the exterior surfaces of air sample cassettes prior to entry.

Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

10.3 Equipment for analysis

10.3.1 Transmission electron microscope

A TEM^[3] operating at an accelerating potential of 80 kV or greater, with a resolution better than 1,0 nm, and a magnification range of approximately $\times 300$ to $\times 100\,000$ shall be used. The ability to obtain a viewing screen magnification of about $\times 100\,000$ is necessary for inspection of fibre morphology; this magnification may be obtained by supplementary optical or electronic enlargement of the screen image. A method for making fibre length and width measurements from the screen image is required. This requirement is often fulfilled through the use of a fluorescent screen with calibrated gradations in the form of circles at 1 cm radius increments, as shown in Figure 1. This design allows lengths and widths of fibre images down to 1 mm width to be measured in increments of 1 mm, regardless of image orientation. Alternatively, electronic display systems with measurement software may be used, provided that the system is calibrated to provide fibre measurements within a variation of $\pm 5\%$ at the magnification in use.

For Bragg angles less than 0,01 rad, the TEM shall be capable of performing ED from an area of $0,6\ \mu\text{m}^2$ or less, selected from an in-focus image at a screen magnification of $\times 20\,000$. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If SAED is used, the performance of a particular instrument may normally be calculated using Formula (1):

$$A = 0,7854 \times \left(\frac{D}{M} + 2000C_s\theta^3 \right)^2 \quad (1)$$

where

- A is the effective SAED area, in square micrometres;
- D is the diameter, in micrometres, of the SAED aperture;
- M is the magnification of the objective lens;
- C_s is the spherical aberration coefficient, in millimetres, of the objective lens;
- θ is the maximum required Bragg angle, in radians.

It is not possible to reduce the effective SAED area indefinitely by the use of progressively smaller SAED apertures, because there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

If zone-axis ED analyses are to be performed, the TEM shall incorporate a goniometer stage which permits the TEM specimen to be either:

- a) rotated through 360° , combined with tilting through at least $+30^\circ$ to -30° about an axis in the plane of the specimen;
- b) tilted through at least $+30^\circ$ to -30° about two perpendicular axes in the plane of the specimen.

The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fibre, the goniometer shall be of a type which permits tilting of the specimen and acquisition of EDXA spectra without changing the specimen holder.

The TEM shall have an illumination and condenser lens system capable of forming an electron probe of diameter less than 250 nm.

Use of an anti-contamination trap around the specimen is recommended if the required instrumental performance is to be obtained.

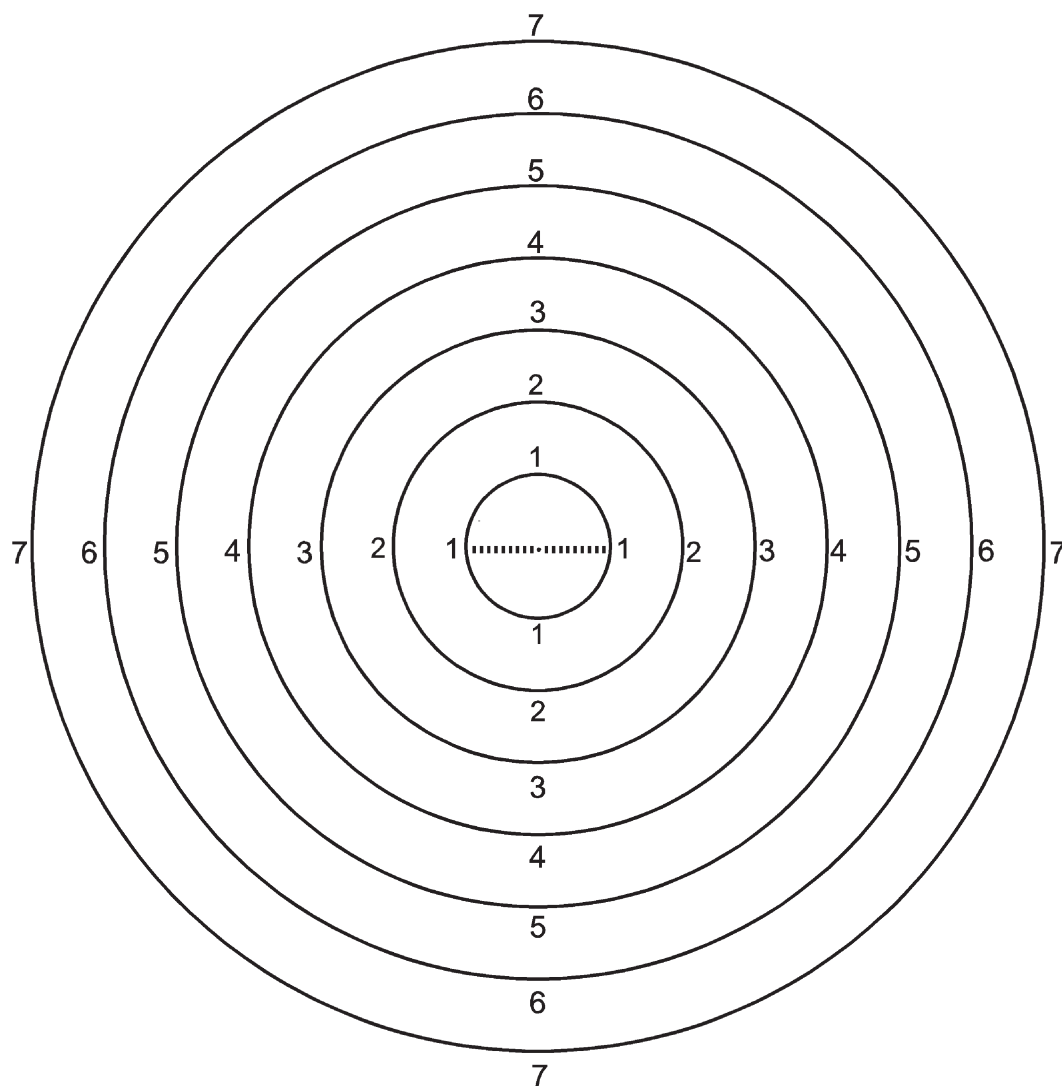


Figure 1 — Example of calibration markings on TEM viewing screen

10.3.2 Energy dispersive X-ray analyser

The TEM shall be equipped with an energy dispersive X-ray analyser capable of achieving a resolution better than 180 eV (FWHM) on the $MnK\alpha$ peak. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of geometrical factors, the required performance of the combination of the TEM and X-ray analyser is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known beam diameter. Solid state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyser shall yield, under routine analytical conditions, a background-subtracted $NaK\alpha$ integrated peak count rate of more than 1 count per second (cps) from a fibre of UICC crocidolite, 50 nm in diameter or smaller, when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1,0.

At a minimum, the EDXA unit shall provide the means for the identification of elemental peaks for qualitative assessment of the asbestos fibre type. For quantitative analysis, the system shall provide means for subtraction of the background, identification of elemental peaks, and calculation of background-subtracted peak areas. For quantitative determination of the asbestos type, a means for calculation of the oxide weight percentages for sodium, magnesium, aluminium, silicon, potassium, calcium, manganese and iron is required.

10.3.3 Plasma asher

For preparation of TEM specimens from MEC filters, a plasma asher^[22], with a radio frequency power rating of 50 W or higher, shall be used to etch the surface of collapsed MEC filters. The asher shall be supplied with a controlled oxygen flow, and shall be modified, if necessary, to provide a valve to control the speed of air admission so that rapid air admission does not disturb particles and fibres from the surface of the filter after the etching step.

It is recommended that filters be fitted to the oxygen supply and the air admission line.

10.3.4 Vacuum coating unit

A vacuum coating unit capable of producing a vacuum better than 0,013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample holder is required which will allow a glass microscope slide to be continuously rotated during the coating procedure.

A mechanism that also allows the rotating slide to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

10.3.5 Sputter coater

A sputter coater with a gold target or a vacuum coating unit with gold evaporation facilities may be used for deposition of gold onto TEM specimens as an integral calibration of ED patterns. Other electron diffraction calibration materials are acceptable. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

10.3.6 Beakers

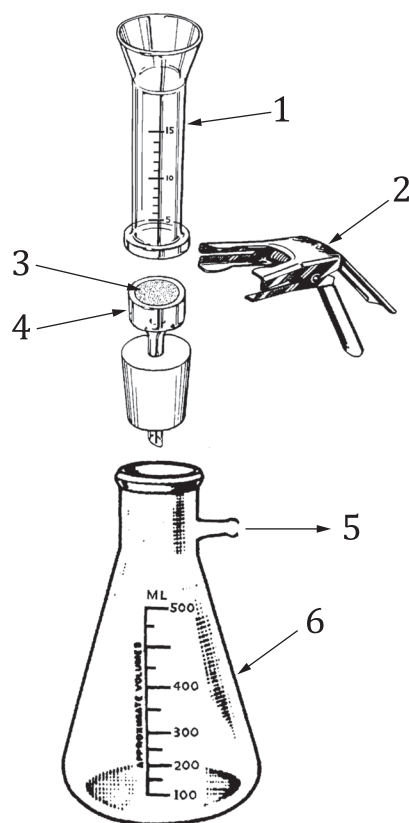
Borosilicate glass, 50 ml beakers are required as containers for plasma ashing of sample collection filters.

10.3.7 Vacuum source

A pump is required to provide a vacuum of at least 20 kPa for the filtration of aqueous dispersions. A water jet pump is suitable.

10.3.8 Glass filtration apparatus

A glass filtration apparatus is required for the filtration of aqueous dispersions. This consists of a filtration base with a porous support for the filter, a reservoir with vertical sides, a clamp to hold the reservoir to the filtration base, and a vacuum flask or filtration manifold. A suitable apparatus is shown in [Figure 2](#).



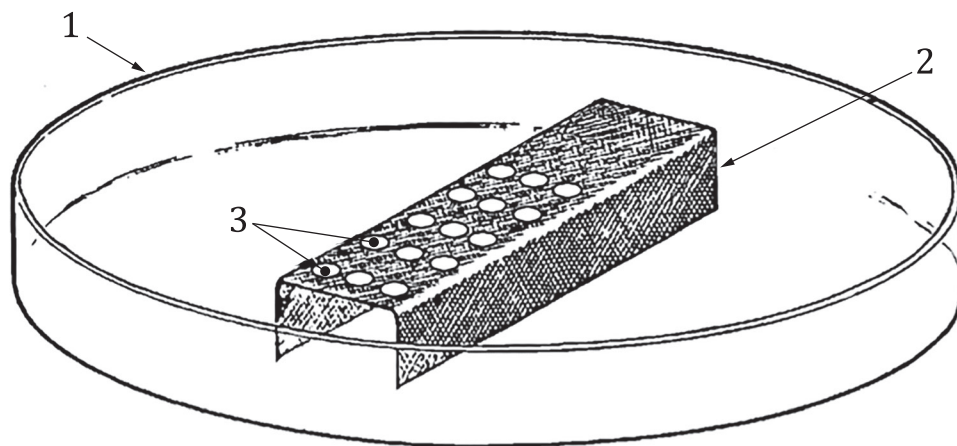
Key

- | | | | |
|---|----------------------|---|-----------------|
| 1 | filtration reservoir | 4 | filtration base |
| 2 | clamp | 5 | vacuum source |
| 3 | porous support | 6 | vacuum flask |

Figure 2 — Example of filtration apparatus

10.3.9 Solvent washer (Jaffe washer)

The purpose of the Jaffe washer^[25] is to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibres and other particles from the filter surface. One design of a washer which has been found satisfactory for various solvents and filter media is shown in [Figure 3](#).



Key

- 1 glass Petri dish (100 mm diameter, 15 mm deep)
- 2 stainless steel mesh bridge (50 mesh)
- 3 electron microscope specimen grids

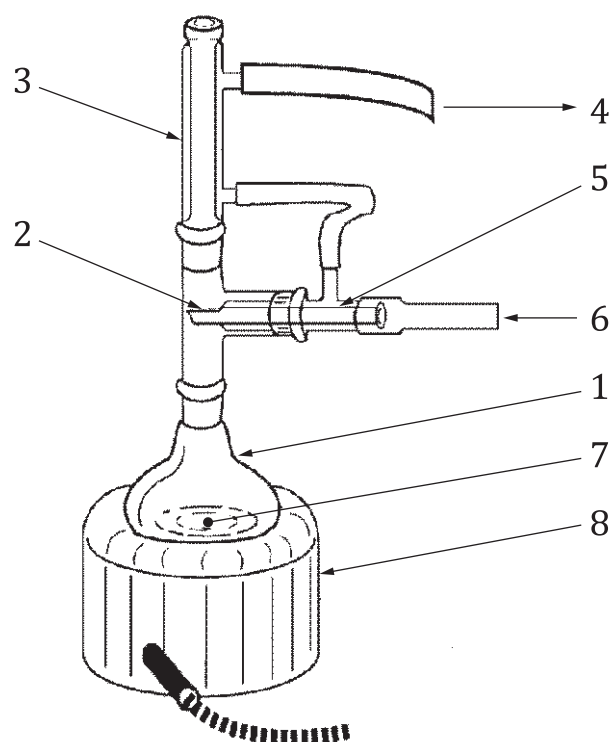
NOTE Solvent is added until the meniscus contacts the underside of the stainless-steel bridge.

Figure 3 — Example of Jaffe washer

In general, either chloroform or a mixture of 80 % 1-methyl-2-pyrrolidone and 20 % 1,2 diaminoethane^[13] has been used for dissolving polycarbonate filters and dimethylformamide or acetone has been used for dissolving MEC or cellulose nitrate filters. The higher evaporation rates of chloroform and acetone require that a reservoir of 10 ml to 50 ml of solvent be used, which may need replenishment during the procedure. Dimethylformamide and 1-methyl-2-pyrrolidone have lower vapour pressures and much smaller volumes of solvent may be used. It is recommended that all washers be used in a fume hood, and when specimens are not being inserted or removed, the Petri dish lid shall be in place during the solvent dissolution. The washer shall be cleaned before it is used for each batch of specimens.

10.3.10 Condensation washer

A condensation washer may be used for rapid dissolution of the filter polymer, and it may be effective if difficulties are experienced in dissolving the filter polymer. A suitable assembly is shown in [Figure 4](#), for use with either acetone or chloroform as the solvent, depending on the type of filter.



Key

- | | |
|---------------|--|
| 1 flask | 5 cold finger |
| 2 specimens | 6 cold water source |
| 3 condenser | 7 solvent |
| 4 water drain | 8 thermostatically-controlled heating mantle |

Figure 4 — Design of condensation washer

Do not use dimethylformamide, 1-methyl-2-pyrrolidone or 1,2-diaminoethane in a condensation washer.

10.3.11 Slide warmer or oven

Use either a slide warmer or an oven for heating slides during the preparation of TEM specimens from MEC or cellulose nitrate filters. It is required to maintain a temperature of 65 °C to 70 °C.

10.3.12 Ultrasonic bath

An ultrasonic bath is necessary for cleaning the apparatus used for TEM specimen preparation.

10.3.13 Carbon grating replica

A carbon grating replica with about 2 000 parallel lines per millimetre shall be used to calibrate the magnification of the TEM.

10.3.14 Calibration specimen grids for EDXA

TEM specimen grids prepared from dispersions of calibration minerals are required for calibration of the EDXA system. The most suitable calibration materials are those that have compositions similar to those of the asbestos minerals being analysed. One such suitable calibration mineral is Kakanui hornblende^[26], which is an amphibole containing all chemical elements relevant in asbestos analysis. Other suitable calibration minerals for individual elements are riebeckite, chrysotile, halloysite,

phlogopite, wollastonite and bustamite. The mineral used for calibration of the EDXA system for sodium shall be prepared using a gold TEM grid.

For qualitative identification of fibres of the regulated asbestos varieties, TEM specimen grids prepared from the reference asbestos samples may be used. The major commercial asbestos varieties exhibit only small variations in composition.

10.3.15 Carbon rod sharpener

The use of necked carbon rods, or equivalent, allows the carbon to be evaporated onto the filters with a minimum of heating.

10.3.16 Disposable tip micropipettes

A disposable tip micropipette, capable of transferring a volume of approximately 30 µl, is necessary for the preparation of TEM specimen grids from MEC filters.

10.3.17 Thermometer

A thermometer, 0 °C to 100 °C range, is required for calibration of the ultrasonic bath.

10.3.18 Stopwatch

A stopwatch is required for calibration of the ultrasonic bath.

10.4 Consumable supplies

10.4.1 Copper or nickel electron microscope grids

Copper or nickel TEM grids with 200 mesh are recommended. Grids which have grid openings of uniform size such that they meet the requirement specified in [12.7.2](#) shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

NOTE Copper TEM grids are slightly attacked by 1,2-diaminoethane. Provided the exposure time is limited to 15 min, this does not affect the specimen preparation in any way. This issue can be avoided by the use of nickel TEM grids.

10.4.2 Gold or nickel electron microscope grids

Gold or nickel TEM grids with 200 mesh are recommended to mount TEM specimens when sodium measurements are required in the fibre identification procedure. Grids which have grid openings of uniform size such that they meet the requirement specified in [12.7.2](#) shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

10.4.3 Aluminium foil

Aluminium foil is required for covering beakers during the plasma ashing and ultrasonic treatment steps. Aluminium foil of 0,015 mm to 0,020 mm thickness is suitable.

10.4.4 Carbon rod electrodes

Spectrochemically pure carbon rods, shall be used in the vacuum coating unit ([10.3.4](#)) during carbon coating of filters.

10.4.5 Routine electron microscopy tools and supplies.

Fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, lens tissue, gold wire, tungsten filaments and other routine supplies are required.

10.4.6 Reference asbestos samples

Asbestos samples are required for preparation of reference TEM specimens of the primary asbestos minerals. Suitable sets of standards are SRM 1866 (chrysotile, crocidolite and amosite) and SRM 1867 (tremolite, actinolite and anthophyllite) from the US National Institute of Standards and Technology (NIST) or from the UK Health and Safety Executive (HSE) [chrysotile (Canada and Zimbabwe), crocidolite, amosite, tremolite, actinolite and anthophyllite]^[34]. SRM 1867 tremolite and actinolite are particularly useful for qualitative discrimination between tremolite and actinolite. The International Mineralogical Association (IMA) has specified that values of the mass fraction ratio $Mg/(Mg + Fe)$ above 0,9 are defined as tremolite, and those below 0,9 are defined as actinolite^{[23][24]}. SRM 1867 tremolite has a value of 0,94, and SRM 1867 actinolite has a value of 0,84, providing reference samples representing compositions just above and just below the IMA boundary. It is important to recognize that the IMA boundary between tremolite and actinolite is only a convention within a continuum of composition in which the iron and magnesium mass fractions vary in a reciprocal manner.

For those laboratories that are unable to obtain either the NIST or the HSE reference asbestos samples, the Union Internationale Contre le Cancer (UICC) standard reference samples of asbestos may be used. These samples were widely distributed internationally, and can still be obtained, although they do not include tremolite or actinolite.

10.4.7 Reference samples of mineral fibres other than asbestos

If there is interest in identification of fibres other than asbestos, reference samples from known sources of other inorganic fibres should be available to assist in their identification.

11 Air sample collection

11.1 Calculation of analytical sensitivity

The desired analytical sensitivity is a parameter that shall be established for the analysis prior to sample collection. The analytical sensitivity is defined as that structure concentration corresponding to the detection of one structure in the analysis. For indirect transfer methods of TEM specimen preparation, it is a function of the volume of air sampled, the active area of the collection filter, the area of the collection filter that is ashed, the proportion of the aqueous dispersion of ash that is filtered, the area of the analytical filter, and the area of the TEM specimen over which structures are counted. The analytical sensitivity, S , is calculated using [Formula \(2\)](#).

$$S = \frac{(A_a \cdot V_d)}{(k \cdot A_g \cdot V_f \cdot F_a \cdot V_s)} \quad (2)$$

where:

- S the analytical sensitivity, expressed in structures/litre;
- A_a the active area of the analytical filter in square millimetres;
- V_d the volume, expressed in millilitres, of water used for dispersal of residual ash;
- k the number of grid openings examined;
- A_g the mean area of grid openings, expressed in square millimetres;

V_f the volume, expressed in millilitres, of aqueous dispersion filtered;

F_a the fraction of sample collection filter ashed;

V_s the volume of air sampled, in litres.

Examples of the number of grid openings required to be examined, using various combinations of sampling and analytical parameters, are shown in [Table 1](#).

11.2 Sample collection procedure

Air samples shall be collected using filter cassettes as described in [10.1.1](#). During static sampling, the filter cassette shall be supported on a stand which is isolated from the vibrations of the pump. The cassette shall be held facing vertically downwards at a height of approximately 1,5 m to 2,0 m above ground/floor level, and connected to the pump with a flexible tube. Personal samples may also be collected with the filter holder attached to the left or right lapel of the individual, or other position near the breathing zone.

Measure the sampling flow-rate at the front end of the cassette, both at the beginning and end of the sampling period, using a calibrated variable area flowmeter temporarily attached to the inlet of the cassette. The mean value of these two measurements shall be used to calculate the total air volume sampled.

The variable area flowmeter shall be cleaned before use to avoid transfer of fibre contamination from the flowmeter to the sample being collected.

Table 1 — Examples of minimum number of grid openings required to achieve a particular analytical sensitivity and limit of detection

Analytical sensitivity (structures/l)	Limit of detection (structures/l)	Volume of air sampled (litres)						
		500	1 000	2 000	3 000	4 000	5 000	10 000
0,1	0,3	937	469	235	157	118	94	47
0,2	0,6	469	235	118	79	59	47	24
0,3	0,9	313	157	79	53	40	32	16
0,4	1,2	235	118	59	40	30	24	12
0,5	1,5	188	94	47	32	24	19	10
0,7	2,1	134	67	34	23	17	14	7
1,0	3,0	94	47	24	16	12	10	5
2,0	6,0	47	24	12	8	6	5	4
3,0	9,0	32	16	8	6	4	4	4
4,0	12	24	12	6	4	4	4	4
5,0	15	19	10	5	4	4	4	4
7,0	21	14	7	4	4	4	4	4
10	30	10	5	4	4	4	4	4

NOTE In [Table 1](#), it is assumed that one half of the sample collection filter is ashed, the volume of water used to disperse the ash is 40 ml, the volume of aqueous dispersion filtered is 34 ml, the active area of the analytical filter is 199 mm², and the TEM grid openings are square with a linear dimension of 100 µm. The limit of detection is defined as the upper 95 % confidence limit of the Poisson distribution for a count of zero structures. In the absence of background, this is equal to 2,99 times the analytical sensitivity. Non-zero backgrounds observed during analysis of blank filters will degrade the limit of detection.

Basic strategies for monitoring environmental sources of airborne asbestos are described in [Annex I](#). After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed with the filter face-upwards for return to the laboratory. Field blank filters shall also be included, as specified in [12.8](#), and submitted to the remaining analytical procedures along with the samples.