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

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

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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](http://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](http://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: [www.iso.org/iso/foreword.html](http://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 10312:1995), 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](http://www.iso.org/members.html).

## Introduction

This document is applicable to the determination 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 sizes are the relevant parameters for evaluation of the inhalation hazards, a fibre counting 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 detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. Airborne asbestos is often found as a mixture of single fibres and more complex, aggregated structures which may or may not be also aggregated with other particles. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if a sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis would become 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 factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of, asbestos fibres is required. The method specified in this document is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. This document requires that a very detailed and logical procedure be used to reduce the subjective aspects of the measurement. The method of data recording specified in this document is designed to allow re-evaluation of the structure counting data as new medical evidence becomes available. All feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion onto 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. However, the procedures specified in this document are designed to minimize the disturbance of the collected particulate material, and the effect of those disturbances that do occur can be evaluated.

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 this document's accuracy and precision.

# Ambient air — Determination of asbestos fibres — Direct 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 method allows determination of the type(s) of asbestos fibres present and also includes measurement of the lengths, widths and aspect ratios of the asbestos structures. The method cannot discriminate between individual fibres of asbestos and elongate fragments (cleavage fragments and acicular particles) from non-asbestos analogues of the same amphibole mineral<sup>[13]</sup>.

## 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

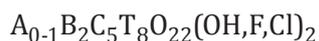
#### **acicular**

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

### 3.2

#### **amphibole**

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



where

A = K, Na;

B = Fe<sup>2+</sup>, Mn, Mg, Ca, Na;

C = Al, Cr, Ti, Fe<sup>3+</sup>, Mg, Fe<sup>2+</sup>;

T = Si, Al, Cr, Fe<sup>3+</sup>, 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°.

**3.3  
amphibole asbestos**

*amphibole* (3.2) in an *asbestiform* (3.5) habit

**3.4  
analytical sensitivity**

calculated airborne *asbestos structure* (3.7) concentration in structures/litre, equivalent to counting of one *asbestos* (3.6) 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.

**3.5  
asbestiform**

specific type of mineral fibrosity in which the *fibres* (3.22) and fibrils possess high tensile strength and flexibility

**3.6  
asbestos**

group of silicate minerals belonging to the serpentine and *amphibole* (3.2) groups, which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong *fibres* (3.22) 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<sup>[9]</sup> may also be found in some products such as vermiculite and talc.

**3.7  
asbestos structure**

individual *fibre* (3.22), or any connected or overlapping grouping of *asbestos* (3.6) fibres or bundles, with or without other particles

**3.8  
aspect ratio**

ratio of length to width of a particle

**3.9  
blank**

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

**3.10  
camera length**

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

**3.11  
chrysotile**

fibrous mineral of the serpentine group, which has the nominal composition:  $Mg_3Si_2O_5(OH)_4$

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.

**3.12****cleavage**

breaking of a mineral along one of its crystallographic directions

**3.13****cleavage fragment**

fragment of a crystal that is bounded by *cleavage* (3.12) faces

Note 1 to entry: Crushing of non-asbestiform amphibole generally yields elongated fragments that conform to the definition of a fibre.

**3.14****cluster**

structure in which two or more *fibres* (3.22), or *fibre bundles* (3.23), are randomly oriented in a connected grouping

**3.15****d-spacing**

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

**3.16****electron diffraction****ED**

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

**3.17****electron scattering power**

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

**3.18****energy dispersive X-ray analysis****EDXA**

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

**3.19****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

**3.20****field blank**

filter cassette that has been taken to the sampling site, opened and then closed

Note 1 to entry: Such a filter is used to determine the background structure count for the measurement.

**3.21****fibril**

single *fibre* (3.22) of *asbestos* (3.6), which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances

**3.22****fibre**

elongated particle that 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.

**3.23**

**fibre bundle**

structure composed of parallel, smaller diameter *fibres* (3.22) attached along their lengths

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

**3.24**

**fibrous structure**

fibre, or connected grouping of *fibres* (3.22), with or without other particles

**3.25**

**habit**

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

**3.26**

**limit of detection**

calculated airborne *fibre* (3.22) 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

**3.27**

**matrix**

structure in which one or more *fibres* (3.22) or *fibre bundles* (3.23), touch, are attached to, or are partially concealed by a single particle or connected group of non-fibrous particles

**3.28**

**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

**3.29**

**phase contrast optical microscopy equivalent fibre**

**PCM equivalent fibre**

*fibre* (3.22) 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

**3.30**

**phase contrast optical microscopy equivalent structure**

**PCM equivalent structure**

*fibrous structure* (3.24) 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

**3.31**

**pixel**

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

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

**3.32**

**primary structure**

*fibrous structure* (3.24) that is a separate entity in the transmission electron microscope image

**3.33**

**replication**

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

**3.34**

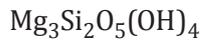
**selected area electron diffraction**

**SAED**

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

**3.35****serpentine**

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

**3.36****structure**

single *fibre* (3.22), *fibre bundle* (3.23), *cluster* (3.14) or *matrix* (3.27)

**3.37****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

**3.38****unopened fibre**

large diameter *asbestos* (3.6) *fibre bundle* (3.23) that has not been separated into its constituent fibrils or *fibres* (3.22)

**3.39****zone-axis**

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

**4 Symbols and abbreviated terms**

eV	electron volt
kV	Kilovolt
l/min	litres per minute
µg	microgram (10 <sup>-6</sup> grams)
µm	micrometre (10 <sup>-6</sup> metre)
nm	nanometre (10 <sup>-9</sup> 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 range of concentration which can be determined is 7 000 structures/mm<sup>2</sup> on the filter. The lower range is dependent on the area of the TEM specimens analysed, but measurement of concentrations lower than 1 structure/mm<sup>2</sup> can be achieved. The air concentrations represented by these values are a function of the volume of air sampled. There is no lower limit to the dimensions of asbestos fibres which 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 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 TEM specimen examined is controlled by the total suspended particulate concentration.

For total suspended particulate concentrations of approximately 10 µg/m<sup>3</sup>, 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 mm<sup>2</sup> 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. If higher total suspended particulate concentrations are present, the volume of air filtered must be reduced in order to maintain an acceptable particulate loading on the filter, leading to a proportionate increase in the analytical sensitivity.

Where this is the case, lower limits of detection can be achieved by increasing the area of the TEM specimens that is examined. 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. The direct analytical method becomes increasingly difficult and imprecise as the general particulate loading of the sample collection filter increases. It is recommended that no more than approximately 25 % of the area of the grid openings be occupied by particulate that is capable of obscuring fibres of interest, which corresponds to approximately 25 µg/cm<sup>2</sup> of filter surface. The dimensions of the airborne particles on the filter and the dimensional range of fibres being evaluated determine the extent to which any asbestos could be overlain and obscured. If the total suspended particulate is largely organic material, the limit of detection can be lowered significantly by using an indirect preparation method.

## 8 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a capillary-pore polycarbonate 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 0,45 µm by means of a battery-powered or mains-powered pump. TEM specimens are prepared from polycarbonate filters by a carbon replication procedure<sup>[11]</sup> 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 of the filter surface, which bridges the openings in the TEM specimen grid, and which supports each particle from the original filter in its original position. Cellulose ester 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<sup>[23]</sup> to ensure that all particles are exposed<sup>[12]</sup>. 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<sup>[26]</sup>.

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 structure count on randomly selected grid openings. In the TEM analysis, electron diffraction (ED)<sup>[7]</sup> is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA)<sup>[6]</sup>. 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 electron diffraction pattern for a selected area, and the qualitative and quantitative energy dispersive X-ray analyses. Confirmation of the identification of chrysotile is done only by quantitative ED, and confirmation of amphibole is done 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 structures that are more complex are referred to as "asbestos structures". A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures. The two codes remove the requirement to interpret the structure counting data from the microscopist 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 and fibres are recorded. The number of asbestos structures found on a known area of the microscope sample, together with the equivalent volume of air filtered through this area, is used to calculate the airborne concentration in asbestos structures/litre of air.

## 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 — Equipment and consumable supplies

#### 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  $\mu\text{m}$  or an MEC or cellulose nitrate filter of maximum pore size 0,45  $\mu\text{m}$ . If only fibres longer than 5  $\mu\text{m}$  are to be included in the measurement, PC filters or MEC filters of maximum pore size 0,8  $\mu\text{m}$  are permitted. Either type of filter shall be backed by a 5  $\mu\text{m}$  pore size MEC or cellulose nitrate filter, and supported by a cellulose pad. If push-fit cassettes are used, when the filters are in position, an elastic 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 specified in [12.7](#) for the presence of asbestos structures before any are used for air sample collection.

#### 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  $\pm 10\%$  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 Some combinations of filter pore size and face velocity can result in distortion of the filter by the differential pressure across the filter.

#### 10.1.3 Stand

For static sampling, 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 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 12.7. 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

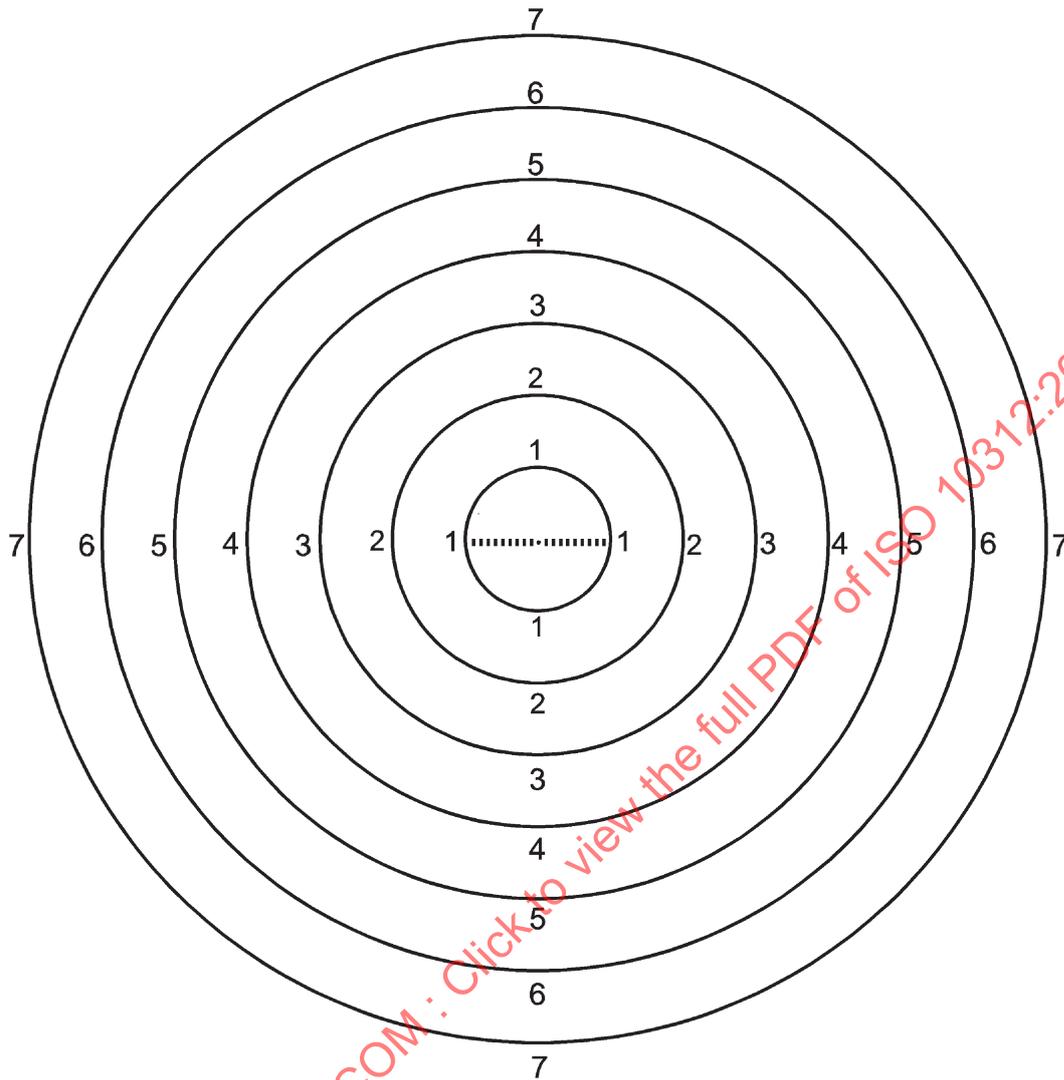
A TEM<sup>[3]</sup> operating at an accelerating potential of 80 kV or greater, with a resolution better than 1,0 nm, and a magnification range of approximately  $\times 300$  to  $\times 100\,000$  shall be used. The ability to obtain a viewing screen magnification of about  $\times 100\,000$  is necessary 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;
- $\theta$  is the maximum required Bragg angle, in radians.



**Figure 1 — Example of calibration markings on TEM viewing screen**

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° to -30° about an axis in the plane of the specimen;
- b) tilted through at least +30° 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.

### 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<sup>[23]</sup>, 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 de-position 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.

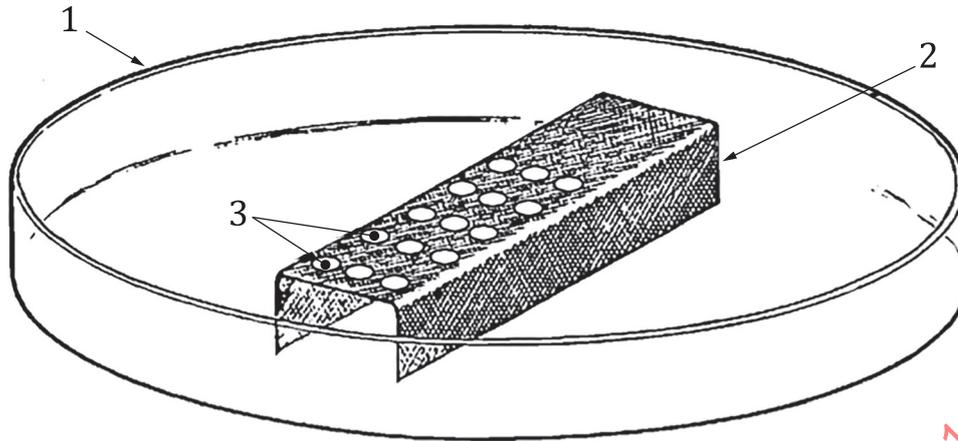
A mechanism that also allows the rotating slide to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping 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 Solvent washer (Jaffe washer)

The purpose of the Jaffe washer<sup>[26]</sup> 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 2](#).



**Key**

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

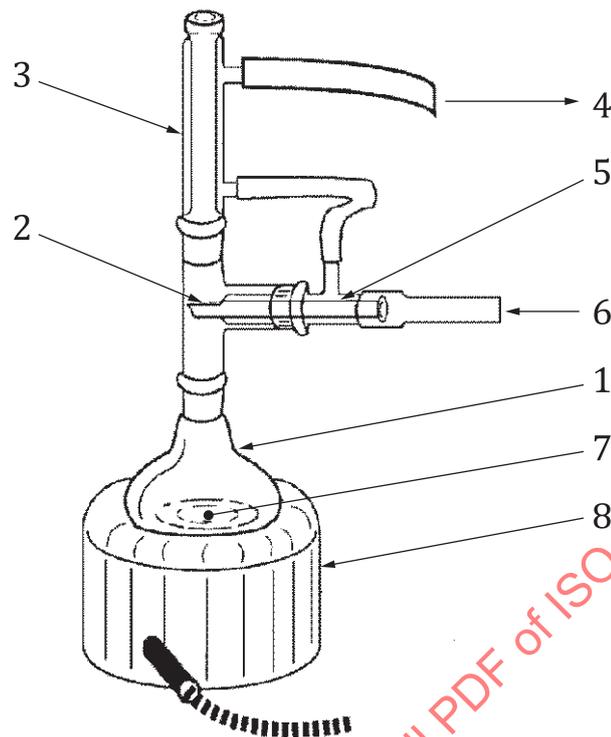
NOTE Solvent is added until the meniscus contacts the underside of the stainless steel bridge.

**Figure 2 — Jaffe washer**

In general, either chloroform or a mixture of 80 % 1-methyl-2-pyrrolidone and 20 % 1,2-diaminoethane<sup>[14]</sup> 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.

**10.3.7 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 3](#), 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 3 — Design of condensation washer**

Dimethylformamide, 1-methyl-2-pyrrolidone or 1,2-diaminoethane shall not be used in a condensation washer.

### 10.3.8 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.

### 10.3.9 Ultrasonic bath

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

### 10.3.10 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.

### 10.3.11 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<sup>[27]</sup>, 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.

#### 10.3.12 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.

#### 10.3.13 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.

### 10.4 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 [12.6.2](#) 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 [12.6.2](#) 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 Carbon rod electrodes

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

#### 10.4.4 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.

#### 10.4.5 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)<sup>[35]</sup>. 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<sup>[24][25]</sup>, 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.

#### 10.4.6 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

The desired analytical sensitivity is a parameter that shall be established for the analysis prior to sample collection. It is defined as the structure concentration corresponding to the detection of one structure in the analysis. For direct transfer methods of TEM specimen preparation, the analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, and the area of the TEM specimen over which structures are counted.

The analytical sensitivity  $S$ , expressed in number of structures per litre, is calculated using [Formula \(2\)](#):

$$S = \frac{A_f}{kA_g V} \quad (2)$$

where

$A_f$  is the active area, in square millimetres, of the sample collection filter;

$A_g$  is the mean area, in square millimetres, of the grid openings examined;

$k$  is the number of grid openings examined;

$V$  is the volume of air sampled, in litres.

If total airborne dust levels are high, it may be necessary to terminate sampling before the required volume has been sampled. If this happens, the analytical sensitivity required can be achieved only by counting structures on more grid openings, or by selective concentration of asbestos structures using an indirect TEM specimen preparation technique. Select the sampling rate and the period of sampling to yield the required analytical sensitivity, as detailed in [Table 1](#). Before air samples are collected, unused filters shall be analysed as described in [12.7](#) to determine the mean asbestos structure count for blank filters.

Air samples shall be collected using filter cassettes ([10.1.1](#)). During static sampling, the cassette shall be supported on a stand or other device ([10.1.3](#)) which is isolated from the vibrations of the pump ([10.1.2](#)). 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 shall be 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 flowmeter ([10.1.4](#)) 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.

Basic strategies for monitoring environmental sources of airborne asbestos are described in [Annex G](#). After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed with the

filter faced upwards for return to the laboratory. Field blank filters shall also be included, as specified in 12.7, and submitted to the remaining analytical procedures along with the samples.

**Table 1 — Examples of the 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 sample (litres)					
		500	1 000	2 000	3 000	4 000	5 000
0,1	0,30	770	385	193	129	97	77
0,2	0,60	385	193	97	65	49	39
0,3	0,90	257	129	65	43	33	26
0,4	1,2	193	97	49	33	25	20
0,5	1,5	154	77	39	26	20	16
0,7	2,1	110	55	28	19	14	11
1,0	3,0	77	39	20	13	10	8
2,0	6,0	39	20	10	7	5	4
4,0	12	20	10	5	4	4	4
5,0	15	16	8	4	4	4	4
7,0	21	11	6	4	4	4	4
10	30	8	4	4	4	4	4

NOTE In Table 1 a collection filter area of 385 mm<sup>2</sup> is assumed, and the TEM grid openings are assumed to be 100 µm square. 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. Backgrounds that are different from zero observed during analysis of blank filters will degrade the limit of detection.

## 12 Procedure for analysis

### 12.1 General

The techniques used to prepare TEM specimens are different for polycarbonate and cellulose ester filters. The preparation method to be used shall be either 12.3 or 12.4, depending on the type of membrane filter used for air sampling. Cleaning of the sample cassettes before they are opened, preparation of the carbon evaporator, criteria for acceptable specimen grids, and the requirement for blank determinations are identical for the two preparation techniques. TEM examination, structure counting, fibre identification and reporting of results are independent of the type of filter or preparation technique used.

The ability to meet the blank sample criteria is dependent on the cleanliness of equipment and supplies. Consider all supplies such as microscope slides and glassware as potential sources of asbestos contamination. It is necessary to wash all glassware before it is used. Wash any tools or glassware which come into contact with the air sampling filters or TEM specimen preparations both before use and between handling of individual samples. Where possible, disposable supplies should be used.

### 12.2 Cleaning of sample cassettes

Asbestos fibres can adhere to the exterior surfaces of air sampling cassettes, and these fibres can be inadvertently transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette before it is transferred to the TEM specimen preparation area.

## 12.3 Direct preparation of TEM specimens from polycarbonate filters

### 12.3.1 Selection of filter area for carbon coating

Use a cleaned microscope slide to support representative portions of polycarbonate filter during the carbon evaporation. Double-coated adhesive tape is used to attach the filter portions to the glass slide. Take care not to stretch the polycarbonate filters during handling. Using freshly cleaned tweezers, remove the polycarbonate filter from the sampling cassette, and place it on to a second cleaned glass microscope slide which is used as a cutting surface. Using a freshly cleaned curved scalpel blade, cut the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. The scalpel blade and tweezers shall be washed and dried between the handling of each filter. Identify the filter portions by writing on the glass slide.

### 12.3.2 Carbon coating of filter portions

Place the glass slide holding the filter portions on the rotation-tilting device, approximately 10 cm to 12 cm from the evaporation source, and evacuate the evaporator chamber (10.3.4) to a vacuum better than 0,013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by a few seconds to allow the electrodes to cool. If evaporation of carbon is too rapid, the strips of polycarbonate filter will begin to curl, and cross-linking of the surface will occur. This cross-linking produces a layer of polymer which is relatively insoluble in organic solvents, and it will not be possible to prepare satisfactory TEM specimens. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30 nm to 50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

### 12.3.3 Preparation of the Jaffe washer

Set up a Jaffe washer<sup>[26]</sup> as shown in Figure 2, and fill the washer with either a mixture of 80 % 1-methyl-2-pyrrolidone (9.3) and 20 % 1,2-diaminoethane (9.4) or chloroform (9.2) to a level where the meniscus contacts the underside of the stainless steel mesh bridge.

### 12.3.4 Placing of specimens in the Jaffe washer

Using a curved scalpel blade, cut three 3 mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion. Select three squares to represent the centre and the periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the stainless-steel bridge of the Jaffe washer.

If a mixture of 20 % 1,2-diaminoethane (ethylene diamine) and 80 % 1-methyl-2-pyrrolidone is used in the Jaffe washer, dissolution of the polycarbonate filters will be completed in 15 min, even if the surface of the filter has been over-heated<sup>[14]</sup>. After a period of 15 min, transfer the stainless-steel bridge into another Petri-dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desired to retain water-soluble particle species on the TEM grids, ethanol may be used instead of distilled water for the second wash.

If chloroform is used in the Jaffe washer, allow the washer to stand for at least 8 h. Remove the mesh and allow the grids to dry.

NOTE 1 It has been found that some polycarbonate filters will not completely dissolve in the Jaffe washer, even after exposure to chloroform for as long as 3 days. This problem is more severe if the surface of the filter was over-heated during the carbon evaporation. It has been found that the problem of residual undissolved filter polymer is best overcome by using a mixture of 80 % 1-methyl-2-pyrrolidone and 20 % 1,2-diaminoethane as the solvent<sup>[14]</sup>.

NOTE 2 If copper TEM grids are in use, avoid exposing them longer than 15 min to the mixture of 1,2-diaminoethane (ethylene diamine) and 80 % 1-methyl-2-pyrrolidone.

## 12.4 Direct preparation of TEM specimens from cellulose ester filters

### 12.4.1 Selection of area of filter for preparation

Using clean tweezers, remove the filter from the filter cassette, and place it on a cleaned microscope slide. Using a clean, curved scalpel blade, cut out a portion of the filter.

### 12.4.2 Preparation of solution for collapsing cellulose ester filters

Mix 35 ml of dimethylformamide (9.5), and 15 ml of glacial acetic acid (9.6) with 50 ml of water (9.1). Store this mixture in a clean bottle. The mixture is stable and suitable for use for up to 3 months after preparation.

### 12.4.3 Filter collapsing procedure

Using a micropipette with a disposable tip (10.3.13), place 15  $\mu\text{l}/\text{cm}^2$  to 25  $\mu\text{l}/\text{cm}^2$  of the solution prepared in 12.4.2 on a cleaned microscope slide, and using the end of the pipette tip, spread the liquid over the area to be occupied by the filter portion. Place the filter portion, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20° so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter portion may be placed on one slide. Place the slide either on a thermostatically controlled slide warmer (10.3.8) at a temperature of 65 °C to 70 °C, or in an oven (10.3.8) at this temperature, for 10 min. The filter collapses slowly to about 15 % of its original thickness. The procedure leaves a thin, transparent polymer film, with particles and fibres embedded in the upper surface.

### 12.4.4 Plasma etching of the filter surfaces

The optimum conditions and time for plasma etching (see 10.3.3) have been determined experimentally from the recovery of fine chrysotile fibrils on 0,8  $\mu\text{m}$  pore size MEC filters. The conditions required in a particular plasma asher shall be established using the procedure specified in Annex A. Place the microscope slide holding the collapsed filter portions in the plasma asher and etch for the time and under the conditions determined. Take care to ensure that the correct conditions are respected. After etching, admit air slowly to the chamber and remove the microscope slide.

Adjust the air admission valve of the plasma asher such that the time taken for the chamber to reach atmospheric pressure exceeds 2 min. Rapid air admission may disturb particulates on the surface of the etched filter.

### 12.4.5 Carbon coating

Coat the microscope slide holding the collapsed filter portions with carbon as specified in 12.3.2.

### 12.4.6 Preparation of the Jaffe washer

Set up a Jaffe washer<sup>[26]</sup> and place the appropriate number of TEM specimen grids on the stainless steel mesh. Fill the Jaffe washer with either dimethylformamide (9.5) or acetone (9.7) to a level where the meniscus contacts the underside of the mesh.

### 12.4.7 Placing of specimens in the Jaffe washer

Using a curved scalpel blade, cut three 3 mm square pieces of carbon-coated MEC filter from the carbon-coated filter portion. Select three squares to represent the centre and the periphery of the active surface of the filter. Place each square of filter, carbon side up, on one of the TEM specimen grids on the stainless-steel bridge of the Jaffe washer. If dimethylformamide is used as the solvent, dissolution of

the filter polymer is complete after 2 h. If acetone is used as the solvent, the filter polymer is normally dissolved after approximately 4 h. After the dissolution period, remove the stainless-steel bridge and allow the grids to dry.

## 12.5 Criteria for acceptable TEM specimen grids

Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Examine the TEM specimen grid in the electron microscope at a sufficiently low magnification ( $\times 300$  to  $\times 1\,000$ ) for complete grid openings to be inspected. Reject the grid in the following cases.

- a) If the TEM specimen has not been cleared of filter medium by the filter dissolution Step. If the TEM specimen exhibits obscured areas due to undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional washing with solvent shall be carried out, or new specimens shall be prepared from the filter. Small areas of undissolved filter medium in a minority of the grid openings are permitted if they do not interfere with the identification and measurement of fibres.
- b) If the sample is overloaded with particulate. If the specimen grid exhibits more than approximately 25 % obscuration on the majority of the grid openings, the specimen shall be designated as overloaded. This filter cannot be analysed satisfactorily using the direct preparation methods because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA, and obscuration of fibres by other particulates may lead to underestimation of the asbestos structure count. If the particle size and electron scattering power of the particulate are such that they do not interfere with the identification and measurement of fibres, higher values of particulate loading are permitted.
- c) If the particulate deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate deposits on the specimen are obviously not uniform from one grid opening to the next, the specimen shall be designated as non-uniform. This condition is a function either of the air sampling conditions or of the fundamental nature of the airborne particulate. Satisfactory analysis of this filter may not be possible unless a large number of grid openings is examined.
- d) If the TEM grid is too heavily loaded with fibrous structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7 000 structures/mm<sup>2</sup>.
- e) If more than approximately 25 % of the grid openings have broken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the asbestos structure count.

**NOTE** If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, an additional carbon coating can be applied to the carbon coated filter, and new specimen grids prepared. The larger particles can often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analysed using the direct preparation methods.

If one or more of the conditions described in b), c), d) or e) exists, it may not be possible to analyse the sample by this method, and analysis by the indirect-transfer method ISO 13794<sup>[2]</sup> should be considered.

TEM specimens prepared from air sample filters collected in some environments may exhibit large numbers of gypsum fibres that have morphology similar to that of amphibole. TEM examination of such specimens for the presence of asbestos fibres is facilitated if these fibres are removed. A procedure for removal of gypsum fibres is described in [Annex H](#).

## 12.6 Procedure for structure counting by TEM

### 12.6.1 General

The examination consists of a count of asbestos structures present on a specified number of grid openings. Fibres are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of structures to be counted depends on the statistical precision

desired. In the absence of asbestos structures, the area of the TEM specimen grids to be examined depends on the required analytical sensitivity. The precision of the structure count depends not only on the total number of structures counted, but also on their uniformity from one grid opening to the next. Additional structure counting will be necessary if greater precision is required.

The estimate of the structure density on the sampling filter shall not be based on the small area represented by one specimen grid. Therefore, grid openings shall be examined on at least two of the three specimen grids prepared. Then, combine the results in the calculation of the structure density. Structure counts for structures  $\geq 0,5 \mu\text{m}$  shall be made at a magnification of approximately  $\times 20\ 000$ , and shall be terminated at the end of the examination of the grid opening on which the 100th asbestos structure is observed, except that the count shall be continued until a minimum of four grid openings have been examined. Otherwise, the structure count shall continue until the specified analytical sensitivity has been achieved.

Magnification requirements for evaluation of fibres and bundles  $>5 \mu\text{m}$  and PCM equivalent fibres are specified in [12.6.6](#) and in [Annex E](#).

The normal range for the number of grid openings which should be examined is from 4 to 20. If insufficient air has been sampled through the filter, the calculation in [Clause 11](#) can indicate that more than 20 grid openings should be examined. When this situation occurs, a larger value of analytical sensitivity may be accepted, unless more grid openings are examined to improve the analytical sensitivity.

#### 12.6.2 Measurement of mean opening area

The mean grid opening area shall be measured for the type of TEM specimen grids in use. The standard deviation of the mean of 10 openings selected from 10 grids should be less than 5 %. Alternatively, or if the 5 % standard deviation criterion cannot be demonstrated, the dimensions of a single typical grid opening on each of the grids examined shall be measured at a calibrated magnification. The mean value of the areas shall be used in the calculation of results.

#### 12.6.3 TEM alignment and calibration procedures

Before structure counting is performed, align the TEM according to instrumental specifications. Calibrate the TEM and EDXA system according to the procedures in [Annex B](#).

#### 12.6.4 Determination of criterion for termination of TEM examination

Before structure counting begins, calculate the area of the specimen to be examined to achieve the selected analytical sensitivity. Calculate the maximum number of grid openings to be examined from [Formula \(3\)](#):

$$k = \frac{A_f}{A_g VS} \quad (3)$$

where:

$k$  is the number of grid openings to be examined, rounded upwards to the next highest integer;

$A_f$  is the area, in square millimetres, of the sample filter;

$A_g$  is the area, in square millimetres, of the TEM specimen grid openings;

$V$  is the volume of air sampled, in litres;

$S$  is the required analytical sensitivity, expressed as the number of structures per litre.

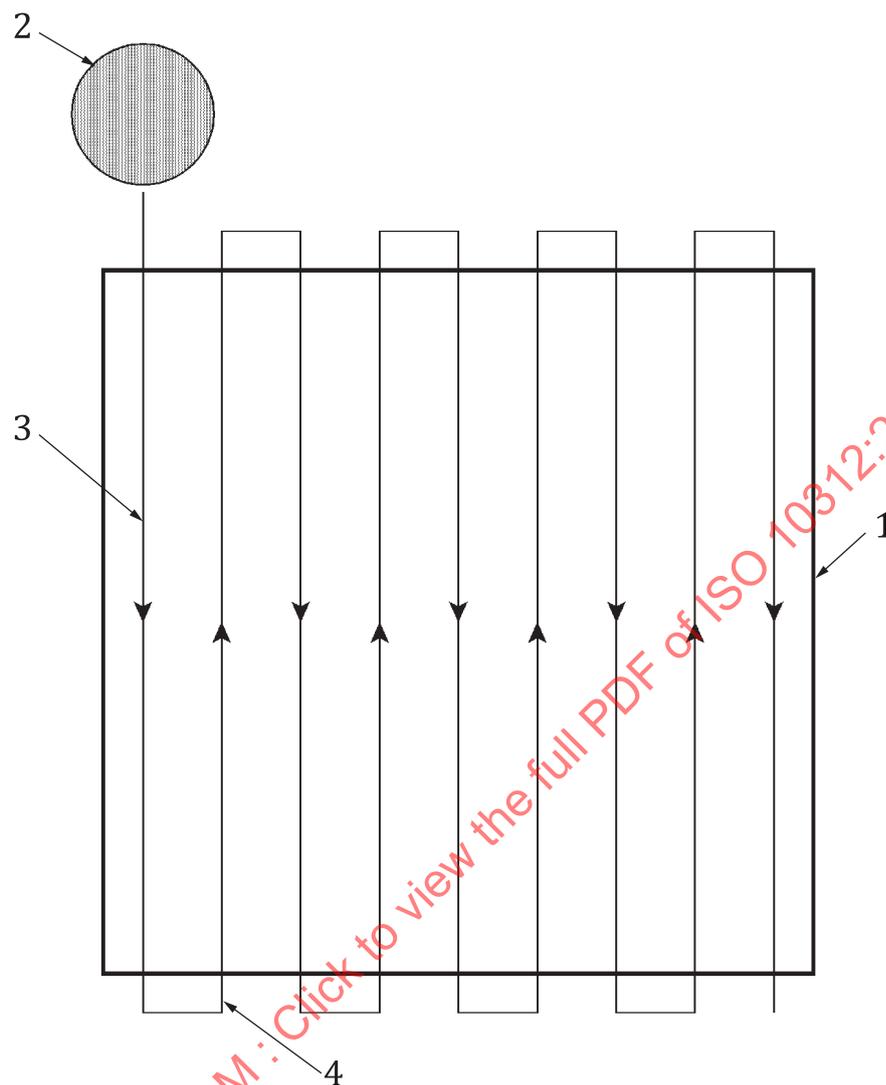
### 12.6.5 General procedure for structure counting and size analysis

Use at least two specimen grids prepared from the filter in the structure count. Select at random several grid openings from each grid, and combine the data in the calculation of the results.

Use a form similar to that shown in [Figure 4](#) to record the structure counting data. Insert the first specimen grid into the TEM. In order to facilitate quality assurance measurements, which require re-examination of the same grid opening by different microscopists, insert the grid into the specimen holder in a standard orientation with the grid bars parallel and perpendicular to the axis of the specimen holder. This will provide scan directions parallel to the edges of the grid opening. Ensure that all microscopists begin scanning at the same starting point on the grid opening, and that they use similar scan patterns. This procedure permits rapid re-location of fibrous structures for further examination if necessary.

Select a typical grid opening and set the screen magnification to the calibrated value specified in [12.6.6](#) for counting structures or fibres in the size range required. Adjust the sample height until the features in the centre of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero. In column 1 of the structure counting form, record the number or letter used to identify the grid. In column 2, record the identification of the particular grid opening. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for fibrous structures, until the opposite side of the grid opening is encountered. Move the image by a pre-determined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in [Figure 5](#). [Figure 5](#) illustrates scanning in an up-down direction; alternatively, the grid opening may be scanned in a left-right direction. When a fibrous structure is detected, assign a sequential number to the primary structure in column 3, perform the identification procedures required as detailed in [Annex D](#), and enter the appropriate compositional classification on the structure counting form in column 5. Assign a morphological classification to the structure according to the procedures in [Annex C](#), and record this in column 6. Measure on the TEM viewing screen the length and width of the image of the primary structure in millimetres, and record these measurements in columns 7 and 8. If the fibre type is determined, enter the name of the mineral in column 9. For a disperse cluster or matrix, assign a compositional classification and a morphological classification to each structure component, measure the length and width, and enter the data in columns 4 to 8. Use column 4 of the structure counting form to tabulate the sequential number of total structures, taking into account structure components. If non-asbestos fibres are observed, note their presence and type, if known. After a fibrous structure has been examined and measured, re-locate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause fibres to be overlooked or counted twice. Continue the examination until the completion of the grid opening on which the 100th primary fibrous structure has been recorded, or until the number of grid openings required to achieve the specified analytical sensitivity, calculated according to 11, have been examined, whichever occurs first. The data shall be drawn approximately equally from a minimum of two grids. Regardless of the value calculated according to 11, fibrous structures on a minimum of 4 grid openings shall be counted.



**Key**

- |   |                   |   |             |
|---|-------------------|---|-------------|
| 1 | grid opening      | 3 | first pass  |
| 2 | TEM field of view | 4 | second pass |

**Figure 5 — Example of scanning procedure for TEM specimen examination**

### 12.6.6 Magnification requirements

For counting structures  $\geq 0,5 \mu\text{m}$  in length, use a fluorescent screen magnification of approximately  $\times 20\,000$ . At this magnification, the image of a single  $0,05 \mu\text{m}$  wide chrysotile fibril is approximately 1 mm in width, and counting can usually be performed without changes of magnification. Consider using a higher magnification for measurement of fibre widths if it is required to measure thin fibres with greater accuracy. Use a magnification of approximately  $\times 10\,000$  for counting of asbestos fibres and bundles longer than  $5 \mu\text{m}$ , or approximately  $\times 5\,000$  if only fibres and bundles longer than  $5 \mu\text{m}$  and within the diameter range  $0,2 \mu\text{m}$  to  $3,0 \mu\text{m}$  are to be counted (PCM equivalent fibres). Use of these lower magnifications for fibres and bundles longer than  $5 \mu\text{m}$  permits larger areas of the TEM specimens to be examined, thus allowing lower analytical sensitivities to be achieved. However, it may be necessary to use a higher magnification for accurate width measurements.

Perform these extended examinations for fibres and bundles longer than  $5 \mu\text{m}$  in accordance with the procedures described in [Annex E](#). Continue the count until completion of the grid opening on

which 100 fibres and bundles have been recorded, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity. Do not include fibres of other materials, such as gypsum, cellulose fibres or filter artefacts, such as undissolved filter strands in the fibre count. Include in the count only those fibres that are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals, in either the original or the extended TEM examination. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

An enhanced imaging system may be used to perform the fibre counts, provided that

- a) equivalence with parallel counts made on the fluorescent screen is demonstrated, and
- b) if magnifications other than those specified in this clause are used, the minimum width specified in the measurements corresponds to at least five pixels in the image used for fibre counting.

## 12.7 Blank and quality control determinations

When possible, before air samples are collected, a minimum of two unused filters from each filter lot of 100 filters shall be analysed to determine the mean background asbestos structure count of the analytical procedure. If the mean background count for all types of asbestos structures is found to be more than 10 structures/mm<sup>2</sup>, or if the mean background fibre count for asbestos fibres longer than 5 µm is more than 0,1 fibre/mm<sup>2</sup>, reject the filter lot.

When performing analyses at very low values of analytical sensitivity, measurement of background asbestos structure count is best achieved by maintaining records of "no asbestos detected" results. The cumulative data may then be used as evidence that the filters in use have background levels of asbestos structures below the analytical sensitivity for any individual analysis.

To ensure that contamination by extraneous asbestos fibres during specimen preparation is insignificant compared with the results reported on samples, establish a continuous programme of blank measurements. At least one field blank shall be processed along with each batch of samples. In addition, at least one unused filter shall be included with every group of samples prepared on one microscope slide.

Initially, and at intervals afterwards, ensure that samples of known asbestos concentrations can be analysed satisfactorily. Since there is a subjective component in the structure counting procedure, re-counts of some specimens shall be made by different microscopists, in order to minimize the subjective effects. Such re-counts provide a means of maintaining comparability between counts made by different microscopists. Variability between and within microscopists and between laboratories shall be characterized. These quality assurance measurements shall constitute approximately 10 % of the analyses. Pairs of results should be within the Poisson 95 % confidence interval for the mean structure count. Provided that it is an infrequent occurrence, a difference of one or two structures outside of the Poisson 95 % confidence interval can be tolerated, particularly for counts of less than 20.

## 12.8 Calculation of results

Calculate the results using the procedures detailed in [Annex F](#). Prior to the TEM examination of the specimens, the level of analysis was specified. Before the results are calculated, the compositional and morphological classifications to be included in the result shall be specified. The between-grid uniformity test specified in [Annex F](#) shall be conducted using the number of primary asbestos structures found on each grid opening, prior to the application of the cluster and matrix counting criteria. The concentration result shall be calculated using the numbers of asbestos structures reported after the application of the cluster and matrix counting criteria.

## 13 Performance characteristics

### 13.1 General

It is important to use this analytical method in conjunction with a continuous quality control programme. The quality control programme should include use of standard samples, blank samples, and both inter- and intra-laboratory analyses.

### 13.2 Interferences and limitations of fibre identification

Unequivocal identification of every chrysotile fibre is not possible, due to both instrumental limitations and the nature of some of the fibres. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the fibre selected. However, there is a possibility of misidentification of fibres for which both the morphologies and the ED patterns are reported on the basis of visual inspection only. Significant possibilities of misidentification occur with halloysite, vermiculite scrolls or palygorskite, all of which can be discriminated from chrysotile by the use of EDXA and by observation of the 0,73 nm (002) reflection of chrysotile in the ED pattern. Rarely, lizardite may exhibit scrolling behaviour, resulting in fibres that resemble chrysotile<sup>[37]</sup>. Although these can be discriminated from chrysotile by detailed electron diffraction analysis, they differ from chrysotile in that they are not sensitive to electron beam heating and the ED pattern does not degrade as occurs with chrysotile.

As in the case of chrysotile fibres, complete identification of every amphibole fibre is not possible due to instrumental limitations and the nature of some of the fibres. Moreover, complete identification of every amphibole fibre is not practical due to the limitations of both time and cost. Particles of a number of other minerals having compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone-axis ED techniques. However, the requirement for quantitative EDXA measurements on all fibres as support for the random orientation ED technique makes misidentification very unlikely, particularly when other similar fibres in the same sample have been identified as amphibole by zone-axis methods. The possibility of misidentification is further reduced with increasing aspect ratio, since it is rare for the minerals with which amphibole may be confused to display an asbestiform habit.

In the TEM, the EDXA spectrum obtained from a fibre will include elemental contributions from attached or overlying particles or even large particles in close proximity to the fibre. These contributions may increase the apparent concentrations of the elements being measured, and often add additional elements to the spectrum. These problems can usually be minimized by careful selection of the part of the fibre being analysed. In other cases, it will be necessary to analyse the interfering particle separately to assess the extent to which it may be contributing to the spectrum obtained from the fibre. Fibres should not be discounted as asbestos without considering the potential contribution to the EDXA spectrum arising from any nearby particles.

### 13.3 Precision and accuracy

#### 13.3.1 Precision

The analytical precision that can be obtained is dependent upon the number of structures counted, and also on the uniformity of the particulate deposit on the analytical filter. Assuming that the structures are randomly deposited on the analytical filter, if 100 structures are counted and the loading is at least 3,5 structures/grid opening, computer modelling of the counting procedure shows that a coefficient of variation of about 10 % can be expected. As the number of structures counted decreases, the precision will also decrease approximately as  $\sqrt{N}$ , where  $N$  is the number of structures counted. In practice, particulate deposits obtained by filtration of ambient air samples are rarely ideally distributed, and it is found that the precision is correspondingly reduced. Degradation of precision is a consequence of several factors, such as:

- a) non-uniformity of the filtered particulate deposit;

- b) distortion of the fibre distribution by application of the structure counting criteria;
- c) variation between microscopists in their interpretation of the fibrous structures;
- d) variation between microscopists in their ability to detect and identify fibres.

The 95 % confidence interval about the mean for a single structure concentration measurement using this analytical method should be approximately  $\pm 25$  % when 100 structures are counted over 10 grid openings.

For the substantially similar method ASTM D6281, a precision statement was determined through statistical analysis of 34 results, from 10 laboratories, on 2 materials. The two sets of air filters were prepared using separate chrysotile and amosite asbestos fibre air dispersions. These results can be found in the current version of ASTM D6281<sup>[10]</sup>.

### 13.3.2 Accuracy

There is no independent method available to determine the accuracy.

**NOTE** It has been demonstrated that, after carbon-coating of polycarbonate membrane filters, particulate material is transferred to the TEM specimens without measurable losses<sup>[15]</sup>. However, if the filters are heavily loaded with particulate, some material can be lost before they are carbon coated. Good comparability between the direct-transfer capillary-pore polycarbonate procedure and the direct-transfer cellulose ester filter procedure has been demonstrated for laboratory-generated aerosols of chrysotile asbestos<sup>[16]</sup>.

### 13.3.3 Inter-laboratory and intra-laboratory analyses

Inter- and intra-laboratory analyses are required in order to monitor systematic errors that can develop among microscopists when using this method. These analyses should be designed to test both the overall method and the performance of individual microscopists. Repeat preparation of TEM grids from different sectors of a filter, followed by examination of the grids by a different microscopist, is a test for reproducibility of the whole method. However, non-uniformity of the particulate deposit on the filter can lead to differences which are not related to the performance of the microscopists. Verified fibre counting (counting of asbestos structures on the same grid opening of a TEM grid by two or more operators, followed by resolution of any discrepancies) may be used both as a training aid and to determine the performance of different microscopists<sup>[32][34]</sup>. The use of indexed TEM grids as defined in [10.4.1](#) and [10.4.2](#) is recommended in order to facilitate re-location of specific grid openings.

### 13.4 Limit of detection

The limit of detection of the method can be varied by choice of the area of the collection filter, the volume of air sampled and the area of the specimen examined in the TEM. It is also a function of the background of asbestos structures on unused filters. A limit of detection shall be quoted for each sample analysis.

**NOTE** In practice, the lowest limit of detection is frequently determined by the total suspended particulate concentration, since each particle on the analytical filter is separated from adjacent ones by a sufficient distance so that the particle can be identified without interference. Particulate loadings greater than about  $25 \mu\text{g}/\text{cm}^2$  on the analytical filters usually preclude preparation of TEM specimens by the direct methods. If the analysis is to be performed with an acceptable expenditure of time, the area of the specimen examined in the TEM for structures of all sizes is limited in most cases to between 10 and 20 grid openings. In typical ambient or building atmospheres, it has been found that an analytical sensitivity of 1 structure/l can be achieved. In some circumstances, where the atmosphere is exceptionally clean, this can be reduced to 0,1 structure/l or lower. For fibres and bundles longer than  $5 \mu\text{m}$ , the reduced magnifications specified permit larger areas of the TEM specimens to be examined in with an acceptable expenditure of time, resulting in proportionately lower limits of detection. If no structures are found in the analysis, the upper 95 % confidence limit can be quoted as the upper bound of the concentration, corresponding to 2,99 times the analytical sensitivity if a Poisson distribution of structures on the filter is assumed. This 95 % confidence limit for zero structures counted is taken as the detection limit. Since there is sometimes contamination of unused sample filters by asbestos structures, this is also taken into account in the discussion of limits of detection.

## 14 Test report

The test report shall include at least items a) to i) and any one or combination of items j), k) and l):

- a) reference to this document, i.e. ISO 10312;
- b) identification of the sample;
- c) the date and time of sampling, and all necessary sampling data (if known);
- d) the date of the analysis;
- e) the identity of the analyst;
- f) all necessary specimen preparation data;
- g) any procedure used not specified in this document or regarded as an optional procedure;
- h) a statement of the minimum acceptable identification category and the maximum identification category attempted (refer to [Tables D.1](#) and [D.2](#));
- i) a statement specifying which identification and structure categories have been used to calculate the concentration values;
- j) option 1:
  - 1) separate concentration values for chrysotile and amphibole structures equal to or longer than 0,5  $\mu\text{m}$ , expressed in asbestos structures/litre;
  - 2) the analytical sensitivity for structures equal to or longer than 0,5  $\mu\text{m}$ , expressed in asbestos structures/litre;
- k) option 2:
  - 3) separate concentration values for chrysotile and amphibole fibres and bundles longer than 5  $\mu\text{m}$ , expressed in asbestos structures/litre;
  - 4) the analytical sensitivity for fibres and bundles longer than 5  $\mu\text{m}$ , expressed in asbestos structures/litre;
- l) option 3:
  - 5) separate concentration values for chrysotile and amphibole PCM equivalent fibres, expressed in asbestos fibres/ml;
  - 6) the analytical sensitivity for PCM equivalent fibres, expressed in asbestos fibres/ml;

Items m) and n) shall be recorded, but the inclusion of them in the test report is optional:

- m) a complete listing of the structure counting data, including grid opening number, structure number, identification category, structure type, length and width of the structure in  $\mu\text{m}$ , the fibre type of the structure and any comments concerning the structure;
- n) compositional data or recorded EDXA spectra<sup>[5]</sup> for the principal varieties of amphibole, if present.

An example of a suitable format for the structure counting data is shown in [Figures 6](#) and [7](#).

**Determination of asbestos fibres by ISO 10312**

**Sample analysis information**

	Laboratory name	Report number	Date
SAMPLE:	Sampling location		
	Exterior sample 2019-03-30		
Air volume:		5 750,0 l	
Area of collection filter:		385,0 mm <sup>2</sup>	
Volume flowrate:		10,0 l/min	
Level of analysis (chrysotile):		CD or CMQ	
Level of analysis (amphibole):		ADQ	
Magnification used for structure counting:		x20 500	
Aspect ratio for fibre definition		5:1	
Mean dimension of grid openings:		95,4 µm	
Initials of analyst		JMW	
Number of grid openings examined:		10	
Analytical sensitivity:		0,736 structures/l	
Number of primary asbestos structures:		13	
Number of asbestos structures counted:		26	
Number of asbestos structures > 5 µm:		7	
Number of asbestos fibres and bundles ≥ 5 µm:		10	
Number of PCM equivalent asbestos structures:		3	
Number of PCM equivalent asbestos fibres:		5	

**Figure 6 — Example of format for reporting sample data**

**SAMPLE ANALYSIS INFORMATION (Page 2 and higher)**

Laboratory name                      Report number                      Date

SAMPLE:    Sampling location

Exterior sample 2019-03-30

**TEM ASBESTOS STRUCTURE COUNT - RAW DATA**

Grid	Grid Opening	Structures		Identification <sup>a</sup>	Structure type	Length $\mu\text{m}$	Width $\mu\text{m}$	Fibre Type	Comments
		primary	total						
A	F4-4	1	1	CD	F	1,7	0,045	Chrysotile	
		2	2	CMQ	B	3,6	0,09	Chrysotile	
		3	3	ADQ	F	4,0	0,15	Crocidolite	
	E3-6	4	4	CD	MC+0	3,5	1,3	Chrysotile	
		E5-1	5		CD	MD43	7,5	5,0	
	5			CD	MB	7,7	0,30	Chrysotile	
	6			CMQ	MF	5,6	0,045	Chrysotile	
	7		CD	MB	5,1	0,30	Chrysotile		
8		CD	MF	1,7	0,045	Chrysotile			
B	F4-1	6		CD	CD+0	6,5	3,0	Chrysotile	
			9	CD	CB	3,5	0,15	Chrysotile	
			10	CD	CF	3,5	0,045	Chrysotile	
		11	CMQ	CR+0	2,6	1,9	Chrysotile		
	G5-1	7		CD	CD31	6,1	3,2	Chrysotile	
			12	CD	CB	5,6	0,30	Chrysotile	
			13	CMQ	CF	4,0	0,045	Chrysotile	
	E4-4	8		CMQ	CB	3,2	0,090	Chrysotile	
			14	CD	B	1,5	0,23	Chrysotile	
9	16	AD	F	8,7	0,15	Unidentified			
C	G4-4	10		CMQ	CD42	25,	5,6	Chrysotile	
			17	CMQ	CB	15,	0,15	Chrysotile	
			18	CMQ	CF	9,4	0,045	Chrysotile	
			19	ADQ	CF	3,6	0,30	Tremolite	
			20	CM	CF	4,2	0,045	Chrysotile	
	E4-4	11		No Fibres					
			ADQ	CD33	9,4	5,5	Amosite		
			21	ADQ	CF	7,1	0,30	Amosite	
	E5-6	11		ADQ	CF	6,2	0,10	Crocidolite	
			22	ADQ	CF	6,2	0,10	Crocidolite	
			23	ADQ	CB	5,1	0,20	Amosite	
			24	ADQ	F	15,3	0,20	Crocidolite	
			25	CMQ	MC10	3,7	2,1	Chrysotile	
F4-1	12	25	CMQ	MC10	3,7	2,1	Chrysotile		
13	26	CD	CC+0	7,4	0,5	Chrysotile			
<sup>a</sup> Identification codes listed in Tables D.1 and D.2									

**Figure 7 — Example of format for reporting structure counting data**

## Annex A (normative)

### Determination of operating conditions for plasma asher

#### A.1 General

During the preparation of TEM specimens from an MEC or cellulose nitrate filter, the spongy structure of the filter is collapsed into a thinner film of polymer by the action of a solvent. Some of the particles on the surface of the original filter become completely buried in the polymer, and the specimen preparation procedure incorporates a plasma etching step<sup>[23]</sup> to oxidize the surface layer of the polymer. Particles buried by the filter collapsing step are then exposed so that they can become subsequently affixed to the evaporated carbon film without altering their position on the original filter. The amount of etching is critical, and individual ashers vary in performance. Therefore, calibrate the plasma asher (see [10.3.3](#)) to give a known amount of etching of the surface of the collapsed filter. This is carried out by adjusting the radio-frequency power output and the oxygen flowrate, and measuring the time taken to completely oxidize an uncollapsed cellulose ester filter with 25 mm diameter of the same type and pore size as the analytical filters.

#### A.2 Procedure

Place an unused cellulose ester filter, with 25 mm diameter, of the same type as that being used, in the centre of a glass microscope slide. Position the slide approximately in the centre of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8 ml/min to 20 ml/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Measure the time required for complete oxidation of the filter. Determine operating parameters which result in complete oxidation of the filter in a period of approximately 15 min. For etching of collapsed filters, these operating parameters shall be used for a period of 8 min.

## Annex B (normative)

### Calibration procedures

#### B.1 Calibration of the TEM

##### B.1.1 Calibration of TEM screen magnification

The electron microscope should be aligned according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica (see 10.3.10). Methods for calibration of the TEM magnification are specified in ISO 29301<sup>[8]</sup>. Adjust the specimen height to the eucentric position before carrying out the calibration. On the fluorescent viewing screen, measure the distance occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Always repeat the calibration after any instrumental maintenance or change of operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates or film. The ratio between these is a constant value for the particular model of TEM.

If an electronic imaging system is in use, calibrate the magnification on the display device.

##### B.1.2 Calibration of ED camera constant

Calibrate the camera constant of the TEM when used in ED mode. Techniques for ED analysis in the TEM are specified in ISO 25498<sup>[2]</sup>. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film with the specimen adjusted to the eucentric position and select ED conditions. Adjust the objective lens current to optimize the pattern obtained, and measure either on the fluorescent viewing screen or on a recorded image the diameters of the innermost two rings. Calculate the radius-based camera constant,  $\lambda \cdot L$ , for both the fluorescent screen and the photographic plate or film, from the relationship shown in [Formula \(B.1\)](#):

$$\lambda \cdot L = \frac{aD}{2,0\sqrt{h^2 + k^2 + l^2}} \quad (\text{B.1})$$

where

$\lambda$  is the wavelength, in nanometres, of the incident electron;

$L$  is the camera length, in mm;

$a$  is the unit cell dimension of gold, in nm (0,407 86 nm);

$h, k, l$  are the Miller indices of the diffracting plane;

$D$  is the diameter of the (hkl) diffraction ring, in mm.

Using gold as the calibration material, the radius-based camera constant is given by:

—  $\lambda \cdot L = 0,117\ 74 \times D \text{ mm} \cdot \text{nm}$  (smallest ring)

—  $\lambda \cdot L = 0,101\ 97 \times D \text{ mm} \cdot \text{nm}$  (second ring)

## B.2 Calibration of the EDXA system

Perform energy calibration of the EDXA system for a low energy and high energy peak regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10 % of the elemental concentration, to be obtained from EDXA spectra of reference silicate minerals containing the elements Na, Mg, Al, Si, K, Ca, Mn, and Fe, and relevant certified reference materials<sup>[18]</sup>. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination which meets the instrumental specifications of 10.3.1 and 10.3.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection being that they should preferably be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine, and that individual small fragments of the minerals are homogeneous in composition within a few percent. Kakanui hornblende<sup>[27]</sup> is a suitable standard for calibration of the EDXA system. This mineral contains all the elements relevant to asbestos analysis, and has been extensively characterized.

Determine the compositions of these standards by electron microprobe analysis or chemical methods. Crush fragments of the same selected mineral standards and prepare filters by dispersal of the crushed material in water and immediate filtration of the suspensions. Prepare TEM specimens from these filters by the procedures described in Clause 12. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable compositional results can be obtained from different instruments.

NOTE 1 The microprobe analyses of the mineral standards are made by conventional techniques, which can be found in the Bibliography<sup>[6][31]</sup>. In summary, the mineral is first embedded in a mount of polymethyl methacrylate or epoxy resin. The mount is then ground and polished to achieve a flat, polished surface of the mineral fragment. This surface is then analysed using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take account of the water concentration in the minerals, which in the case of chrysotile amounts to 13 % by mass. This water content can vary due to losses in the vacuum system.

Aqueous suspensions of mineral standards should be filtered immediately after preparation, since alkali and alkali earth metals can be partially leached from minerals containing these elements.

Express the results of the electron microprobe analyses as atomic or weight percentage ratios relative to silicon. X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, can then be used to calculate the relationship between peak area ratio and atomic or weight percentage ratio. The technique was described by Cliff and Lorimer<sup>[18]</sup>.

The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus, mass absorption and fluorescence effects are negligible. In a silicate mineral specimen containing element  $i$ , the relationship shown in Formula (B.2) can be used to perform quantitative analyses in the TEM:

$$\frac{C_i}{C_{Si}} = k \times \frac{A_i}{A_{Si}} \quad (\text{B.2})$$

where

$C_i$  is the concentration or atomic proportion of element  $i$ ;

$C_{Si}$  is the concentration or atomic percentage of silicon;

$A_i$  is the elemental integrated peak area for element  $i$ ;

$A_{Si}$  is the elemental integrated peak area for silicon;

$k_i$  is the  $k$ -ratio for element  $i$  relative to silicon (a constant).

NOTE For a particular instrumental configuration and a particular particle size, the value of  $k_i$  is constant.

Optionally, the accuracy of the Cliff and Lorimer technique may be increased by incorporating correction for the particle size effect on peak area ratios<sup>[31]</sup>. This is done by determination of separate values of the constant  $k_i$  for different ranges of fibre diameter. Suitable ranges of fibre diameter are <0,25  $\mu\text{m}$ , 0,25  $\mu\text{m}$  to 0,5  $\mu\text{m}$ , 0,5  $\mu\text{m}$  to 1,0  $\mu\text{m}$ , and >1,0  $\mu\text{m}$ . It is recommended that 20 EDXA measurements be made for each range of fibre diameter

Insert the TEM grid into the TEM, obtain an image at the calibrated higher magnification of about  $\times 20\,000$ , and adjust the specimen height to the eucentric point. If the X-ray detector is a side-entry variety, tilt the specimen towards the X-ray detector. Select an isolated fibre or particle less than 0,5  $\mu\text{m}$  in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centred on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. All background-subtracted peak areas used for calibration shall exceed 400 counts.

Repeat this procedure for 20 particles of each mineral standard. Reject analyses of any obviously foreign particles. Calculate the arithmetic mean concentration to peak area ratio,  $k_i$  ( $k$ -ratio), for each specified element of each mineral standard, and also for each of the fibre diameter ranges if applicable. Periodic routine checks shall be carried out to ensure that there has been no degradation of the detector performance. These  $k$ -ratios are used to calculate the elemental concentrations of unknown fibres, using the Cliff and Lorimer relationship.

If the fibre identification requirements of the analysis are limited to identification of the primary six asbestos varieties (chrysotile, crocidolite, amosite, tremolite, actinolite and anthophyllite), calibration of the EDXA system may be restricted to collection of a reference EDXA spectrum from each of these asbestos varieties. Fibre identification during sample analysis is then based on comparison of the EDXA spectrum obtained from each fibre with these reference spectra. Examples of reference spectra are shown in [Annex D](#).

## Annex C (normative)

### Structure counting criteria

#### C.1 General

In addition to isolated fibres, other assemblages of particles and fibres frequently occur in air samples. Groupings of asbestos fibres and particles, referred to as “asbestos structures”, are defined as fibre bundles, clusters and matrices. The numerical result of a TEM examination depends strongly on whether the analyst assigns such an assemblage of fibres as a single entity, or as the estimated number of individual fibres which form the assemblage. It is therefore important that a logical system of counting criteria be defined, so that the interpretation of these complex structures is the same for all analysts, and so that the numerical result is meaningful. Imposition of specific structure counting criteria generally requires that some interpretation, partially based on uncertain health effects information, be made of each asbestos structure found. It is not the intention of this document to make any interpretations based on health effects, and it is intended that a clear separation shall be made between recording of structure counting data, and later interpretation of those data. The system of coding specified in this document permits a clear morphological description of the structures to be recorded in a concise manner suitable for later interpretation, if necessary, by a range of different criteria, without the necessity for re-examination of the specimens. In particular, the coding system is designed to permit recording of the dimensions of each complex fibrous structure, and also whether these structures contain fibres longer than 5 µm. This approach permits later evaluations of the data to include considerations of particle respirability and comparisons with historical indices of asbestos exposure. Examples of the various types of morphological structure, and the manner in which these shall be recorded, are shown in [Figure C.1](#).

#### C.2 Structure definitions and treatment

##### C.2.1 General

Each fibrous structure that is a separate entity shall be designated as a primary structure. Each primary structure shall be designated as a fibre, bundle, cluster or matrix. These structures are discussed in [C.2.2](#) to [C.2.9](#).

##### C.2.2 Fibre

Any particle with parallel or stepped sides, of minimum length 0,5 µm, and with an aspect ratio of 5:1 or greater, shall be defined as a fibre. For chrysotile asbestos, the single fibril shall be defined as a fibre. A fibre with stepped sides shall be assigned a width equal to the average of the minimum and maximum widths. This average shall be used as the width in determination of the aspect ratio.

##### C.2.3 Bundle

A grouping composed of apparently attached parallel fibres shall be defined as a bundle, with a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may be any value, provided that it contains individual constituent fibres having aspect ratios equal to or greater than 5:1. Bundles may exhibit diverging fibres at one or both ends. A bundle may comprise an unopened fibre bundle in which the fibrils have never been separated, or a parallel assembly of fibrils that have come together in which no constituent fibril extends to the full length of the bundle.

### C.2.4 Cluster

An aggregate of two or more randomly oriented fibres, with or without bundles, shall be defined as a cluster. Clusters occur as two varieties:

- a) disperse cluster (type D): a disperse and open network, in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured;
- b) compact cluster (type C): a complex and tightly bound network, in which one or both ends of each individual fibre or bundle are obscured, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

In practice, clusters can occur in which the characteristics of both types of cluster occur in the same structure. Where this occurs, the structure should be assigned as a disperse cluster, and then a logical procedure should be followed by recording structure components according to the counting criteria. The procedure for treatment of clusters is illustrated by examples in [Figure C.2](#).

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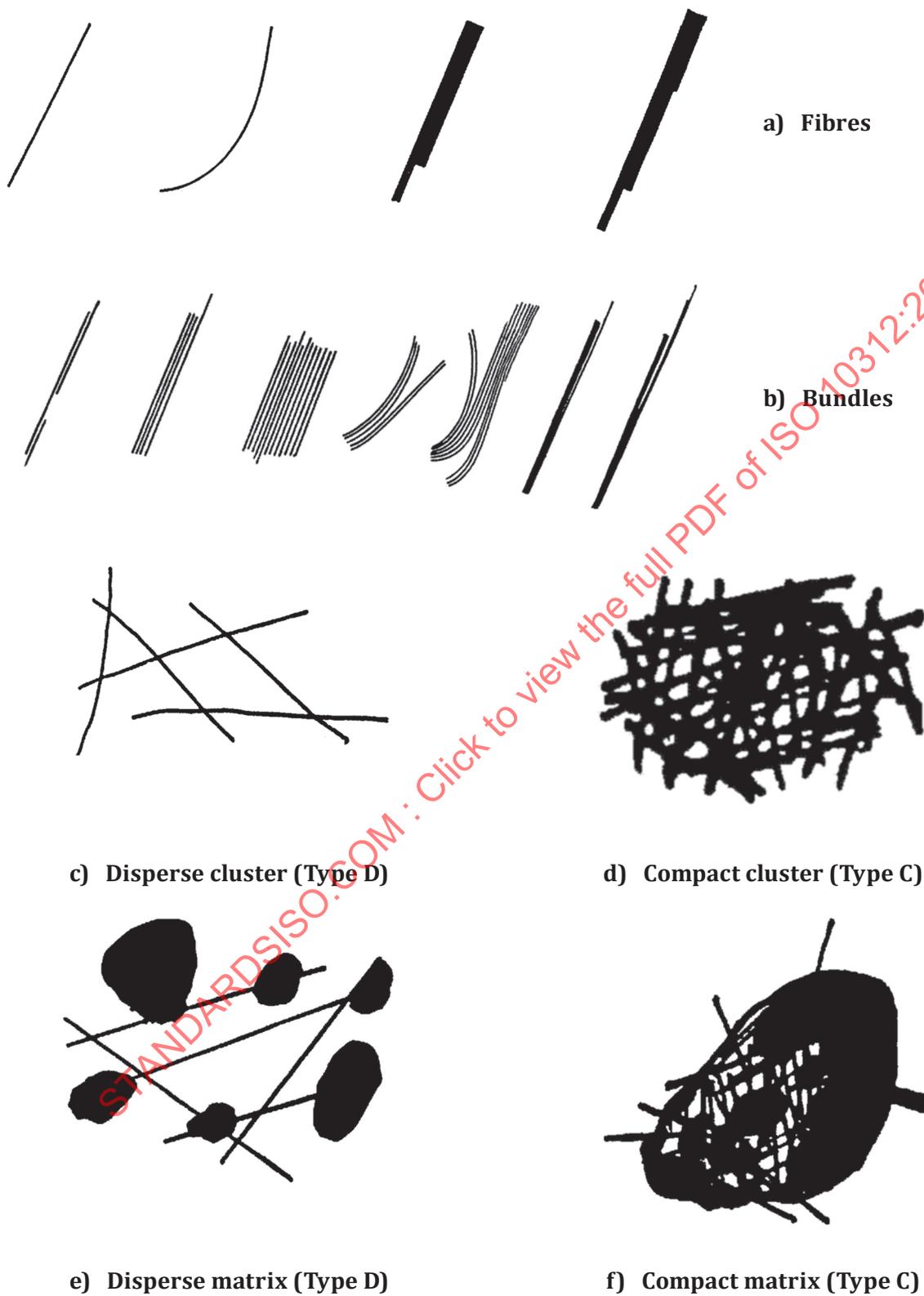
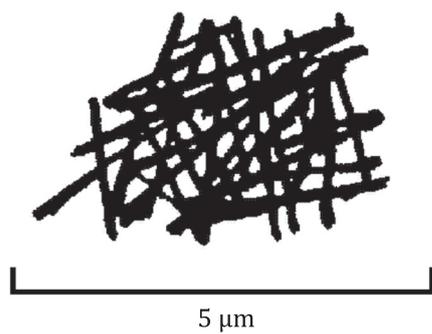
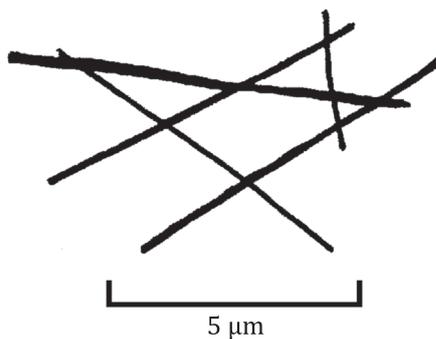


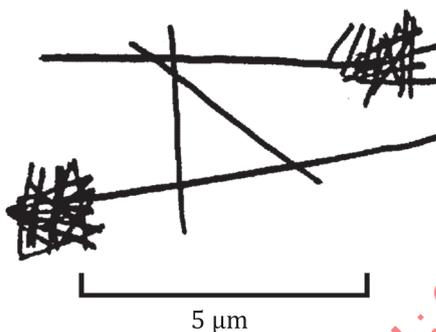
Figure C.1 — Fundamental morphological structure types



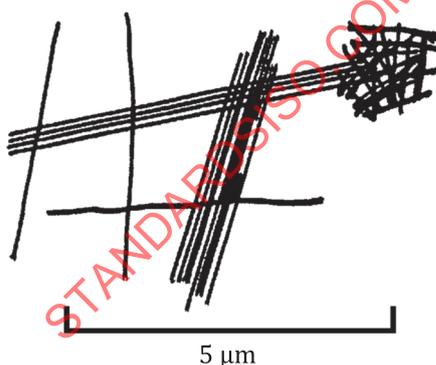
Count as 1 compact cluster (all fibres shorter than 5 μm)  
Record as CC+0



Count as a disperse cluster consisting of 5 fibres, 4 of which are longer than 5 μm  
Record as CD54, followed by 5 fibres each recorded as CF



Count as a disperse cluster consisting of 4 fibres, 2 of which are longer than 5 μm, and 2 cluster residuals  
Record as CD+2, followed by 4 fibres each recorded as CF and 2 cluster residuals each recorded as CR+0



Count as a 1 disperse cluster consisting of 3 fibres, 2 bundles, 1 of which is longer than 5 μm, and 1 cluster residual containing more than 9 fibres  
Record as CD+1, followed by 3 fibres each recorded as CF, 2 bundles each recorded as CB, and one cluster residual recorded as CR+0

Figure C.2 — Examples of recording of complex asbestos clusters

### C.2.5 Matrix

One or more fibres, or fibre bundles, may be attached to, or partially concealed by, a single particle or group of overlapping non-fibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles which are attached to fibres, and those which have by chance overlapped in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibres on the filter.

Since a matrix structure may involve more than one fibre, it is important to define in detail how matrices shall be counted. Matrices exhibit different characteristics, and two types can be defined:

- a) disperse matrix (type D): a structure consisting of a particle or linked group of particles, with overlapping or attached fibres or bundles in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured;
- b) compact matrix (type C): a structure consisting of a particle or linked group of particles, in which fibres or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

In practice, matrices can occur in which the characteristics of both types of matrix occur in the same structure. Where this occurs, the structure should be assigned as a disperse matrix, and then a logical procedure should be followed by recording structure components according to the counting criteria. Examples of the procedure which shall be followed are shown in [Figure C.3](#).

### C.2.6 Asbestos structure larger than 5 µm

Any fibre, bundle, cluster or matrix for which the largest dimension exceeds 5 µm. Asbestos structures larger than 5 µm do not necessarily contain asbestos fibres or bundles longer than 5 µm.

### C.2.7 Asbestos fibre or bundle longer than 5 µm

An asbestos fibre of any width, or bundle of such fibres, which has a length exceeding 5 µm.

### C.2.8 PCM equivalent structure

Any fibre, bundle, cluster or matrix with an aspect ratio of 3:1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. PCM equivalent structures do not necessarily contain fibres or bundles longer than 5 µm, or PCM equivalent fibres.

### C.2.9 PCM equivalent fibre

Any particle with parallel or stepped sides, with an aspect ratio of 3:1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. For chrysotile, PCM equivalent fibres will always be bundles.

## C.3 Other structure counting criteria

### C.3.1 Structures which intersect grid bars

Count a structure which intersects a grid bar for two sides of the grid opening only, as illustrated in [Figure C.4](#). Record the dimensions of the structure such that the obscured portions of components are taken to be equivalent to the unobscured portions, as shown by the broken lines in [Figure C.4](#). For example, the length of a fibre intersecting a grid bar is taken to be twice the unobscured length. Structures intersecting either of the other two sides shall not be included in the count.

### C.3.2 Fibres which extend outside the field of view

During scanning of a grid opening, count fibres which extend outside the field of view systematically, so as to avoid double-counting. In general, a rule should be established so that fibres extending outside the field of view in only two quadrants are counted. The procedure is illustrated by [Figure C.5](#). Measure the length of each such fibre by moving the specimen to locate the other end of the fibre, and then return to the original field of view before continuing to scan the specimen. Fibres without terminations within the field of view shall not be counted.

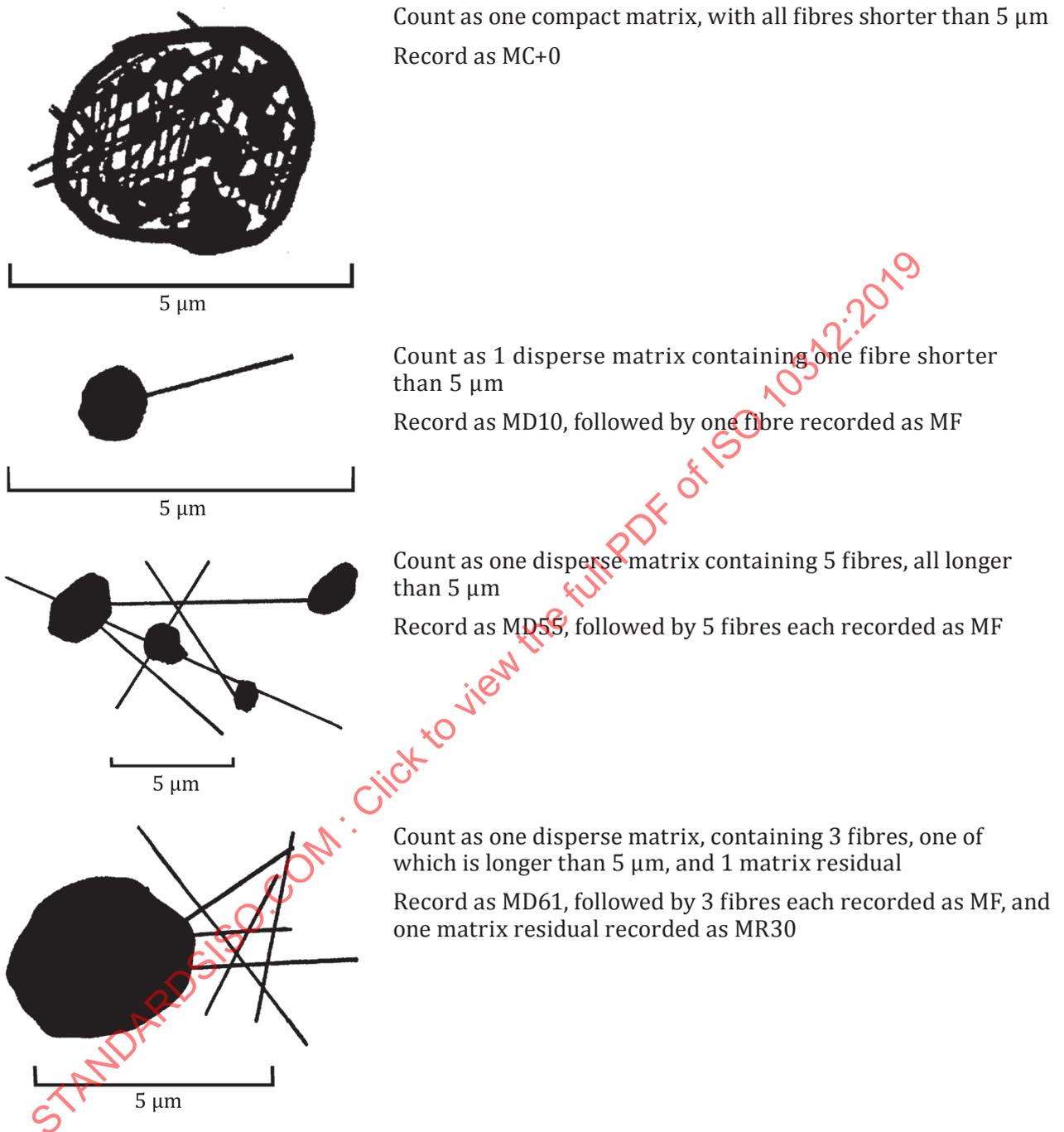
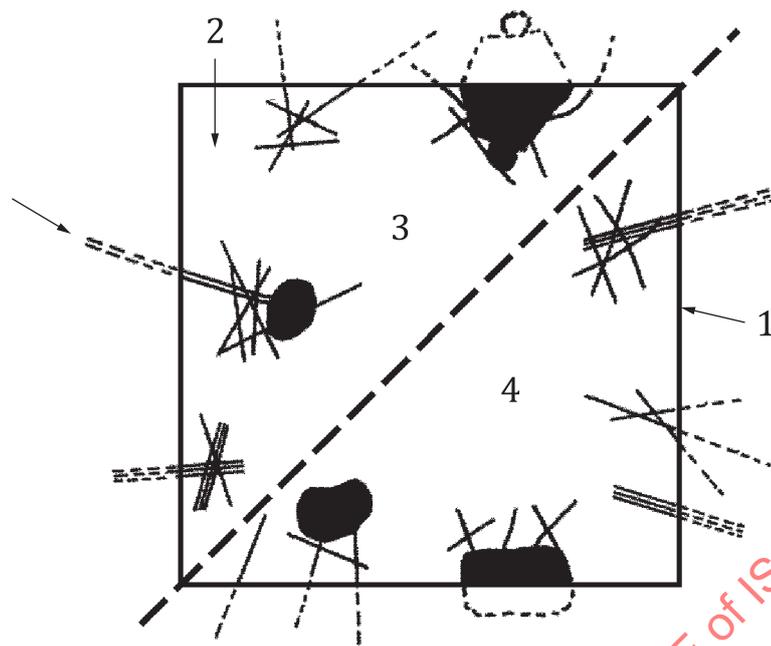


Figure C.3 — Examples of recording of complex asbestos matrices

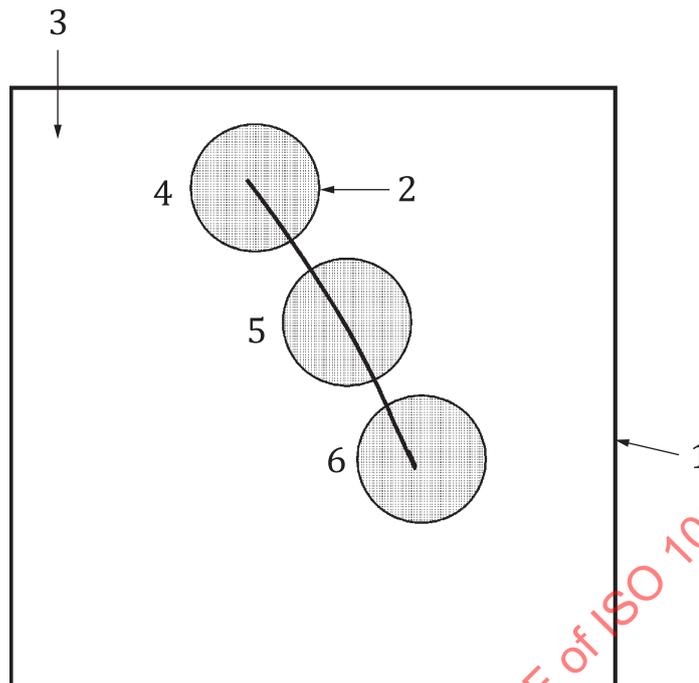


**Key**

- |   |                |   |              |
|---|----------------|---|--------------|
| 1 | grid opening   | 3 | do not count |
| 2 | scan direction | 4 | count        |

**Figure C.4 — Example of counting of structures which intersect grid bars**

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**Key**

1	grid opening	4	count
2	TEM field of view	5	do not count
3	scan direction	6	count

**Figure C.5 — Example of counting of fibres which extend outside the field of view**

## C.4 Procedure for data recording

### C.4.1 General

The morphological codes specified are designed to facilitate computer data processing, and to allow recording of a complete representation of the important features of each asbestos structure. The procedure requires that the microscopist classify each primary fibrous structure into one of the four fundamental categories: fibres, bundles, clusters and matrices.

### C.4.2 Fibres

On the structure counting form, a fibre as defined in [C.2.2](#) shall be recorded by the designation “F”. If the fibre is a separately counted part of a cluster or matrix, the fibre shall be recorded by the designation “CF” or “MF”, depending on whether it is a component of a cluster or matrix.

### C.4.3 Bundles

On the structure counting form, a bundle as defined in [C.2.3](#) shall be recorded by the designation “B”. If the bundle is a separately counted part of a cluster or matrix, the bundle shall be recorded by the designation “CB” or “MB”, depending on whether it is a component of a cluster or matrix.

### C.4.4 Disperse clusters (type D)

On the structure counting form, an isolated cluster of type D as defined in [C.2.4](#) shall be recorded by the designation “CD”, followed by a two-digit number. The first digit represents the analyst’s estimate of the total number of fibres and bundles comprising the structure. The digit shall be from 1 to 9, or designated as “+” if there are estimated to be more than 9 component fibres or bundles. The second digit

shall represent, in the same manner, the total number of fibres and bundles longer than 5 µm contained in the structure. The overall dimensions of the cluster in two perpendicular directions representing the maximum dimensions shall be recorded. In order of decreasing length, up to 5 component fibres or bundles shall be separately recorded, using the codes "CF" (cluster fibre) and "CB" (cluster bundle). If, after accounting for prominent component fibres and bundles, a group of clustered fibres remains, this shall be recorded as "CR" (cluster residual). If the remaining clustered fibres are present as more than one localized group, it may be necessary to record more than one cluster residual. Do not record more than 5 cluster residuals for any cluster. A cluster residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall cluster. Optionally, if the number of component fibres and bundles in either the original cluster or the cluster residual is outside of the range of 1 to 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

#### C.4.5 Compact clusters (type C)

On the structure counting form, an isolated cluster of type C as defined in C.2.4 shall be recorded by the designation "CC", followed by a two-digit number. The two-digit number describing the numbers of component fibres and bundles shall be assigned in the same manner as for clusters type D. The overall dimensions of the cluster in two perpendicular directions shall be recorded in the same manner as for clusters type D. By definition, the constituent fibres and bundles of compact clusters cannot be separately measured, therefore no separate tabulation of component fibres or bundles can be made.

#### C.4.6 Disperse matrices (type D)

On the structure counting form, an isolated matrix of type D as defined in C.2.5 shall be recorded by the designation "MD", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters type D. In order of decreasing length, up to 5 component fibres or bundles shall be separately recorded, using the codes "MF" (matrix fibre) and "MB" (matrix bundle). If, after accounting for prominent component fibres and bundles, matrix material containing asbestos fibres remains, this shall be recorded as "MR" (matrix residual). If the remaining matrix fibres are present as more than one localized group, it may be necessary to record more than one matrix residual. Do not record more than 5 matrix residuals for any matrix. A matrix residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall matrix. Optionally, if the number of component fibres and bundles in either the original matrix or the matrix residual is outside of the range of 1 to 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

#### C.4.7 Compact matrices (type C)

On the structure counting form, an isolated matrix of type C as defined in C.2.6 shall be recorded by the designation "MC", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters type D. By definition, the constituent fibres and bundles of compact matrices cannot be separately measured, therefore no separate tabulation of component fibres or bundles can be made.

#### C.4.8 Procedure for recording of partially obscured fibres and bundles

The proportion of the length of a fibre or bundle that is obscured by other particulate shall be used as the basis for determining whether a fibre or bundle is to be recorded as a separate component or is to be considered as a part of a matrix type C or part of a matrix residual. If the obscured length could not possibly be more than one third of the total length, the fibre or bundle shall be considered a prominent feature to be separately recorded. The assigned length for each such partially-obscured fibre or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Fibres or bundles which appear to cross the matrix, and for which both ends can be located approximately, shall be included in the maximum count of 5 and recorded according to the counting criteria as separate fibres or bundles. If the obscured length could be more than one third of

the total length, the fibre or bundle shall be considered as a part of a compact matrix type C or part of a matrix residual.

### C.5 Special considerations for counting PCM equivalent fibres

Use 3:1 as the minimum aspect ratio for counting PCM equivalent fibres. This aspect ratio definition is required in order to achieve comparability of the results for this size range of fibre with historical optical measurements<sup>[1][28][29]</sup>, but use of this aspect ratio definition does not significantly affect the ability to interpret the whole fibre size distribution in terms of a minimum 5:1 aspect ratio. Some applications may require that a count be made of PCM equivalent fibres only. The coding system permits discrimination between PCM equivalent fibres that contain fibres and bundles longer than 5 µm and those that do not.

NOTE In general, clusters and matrices will yield fewer components as the minimum dimensions specified for countable fibres are increased. Thus, it could be found that a particular structure yields a higher number of component fibres in a count for all fibre sizes than it does at a reduced magnification when only fibres and bundles longer than 5 µm are being counted. However, the requirement that component fibres and bundles be recorded in decreasing length order ensures that the data are consistent for a particular structure, regardless of the size category of fibres being counted and the magnification in use.

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## Annex D (normative)

### Fibre identification procedure

#### D.1 General

The criteria used for identification of asbestos fibres may be selected, depending on the intended use of the measurements. In some circumstances, there can be a requirement that fibres shall be unequivocally identified as a specific mineral species. In other circumstances there can be sufficient knowledge about the sample that rigorous identification of each fibre need not be carried out. The time required to perform the analysis, and therefore the cost of analysis, can vary widely depending on the identification criteria which are considered to be sufficiently definitive. The combination of criteria considered definitive for identification of fibres in a particular analysis shall be specified before the analysis is made, and this combination of criteria shall be referred to as the "Level" of analysis. Various factors related to instrumental limitations and the character of the sample may prevent satisfaction of all the specified fibre identification criteria for a particular fibre. Therefore, a record shall be made of the identification criteria which were satisfied for each suspected asbestos fibre included in the analysis. For example, if both ED and EDXA were specified to be attempted for definitive identification of each fibre, fibres with chrysotile morphology which, for some reason, do not give an ED pattern but which do yield an EDXA spectrum corresponding to chrysotile, are categorized in a way which conveys the level of confidence to be placed in the identification.

#### D.2 ED and EDXA techniques

##### D.2.1 General

Initially classify fibres into two categories on the basis of morphology: those fibres with tubular morphology, and those fibres without tubular morphology. Conduct further analysis of each fibre using ED and EDXA methods. The following procedures should be used when fibres are examined by ED and EDXA.

The crystal structures of some mineral fibres, such as chrysotile, are easily damaged by the high current densities required for EDXA examination. Therefore, investigation of these sensitive fibres by ED shall be completed before attempts are made to obtain EDXA spectra from the fibres. When more stable fibres, such as the amphiboles, are examined, EDXA and ED may be used in either order.

##### D.2.2 ED techniques

The ED technique can be either qualitative or quantitative. Qualitative ED consists of visual examination, without detailed measurement, of the general characteristics of the ED pattern obtained on the TEM viewing screen from a randomly oriented fibre. ED patterns obtained from fibres with cylindrical symmetry, such as chrysotile, do not change when the fibres are tilted about their axes, and patterns from randomly oriented fibres of these minerals can be interpreted quantitatively. For fibres which do not have cylindrical symmetry, only those ED patterns obtained when the fibre is oriented with a principal crystallographic axis closely parallel with the incident electron beam direction can be interpreted quantitatively. This type of ED pattern shall be referred to as a zone-axis ED pattern. In order to interpret a zone-axis ED pattern quantitatively, it shall be recorded photographically and its consistency with known mineral structures shall be checked. A computer program may be used to compare measurements of the zone-axis ED pattern with corresponding data calculated from known mineral structures. The zone-axis ED pattern obtained by examination of a fibre in a particular orientation can be insufficiently specific to permit unequivocal identification of the mineral fibre, but it is often possible to tilt the fibre to another angle and to record a different ED pattern corresponding to

another zone-axis. The angle between the two zone-axes can also be checked for consistency with the structure of a suspected mineral.

For visual examination of the ED pattern, the camera length of the TEM should be set to a low value of approximately 250 mm and the ED pattern then should be viewed through the binoculars. This procedure minimizes the possible degradation of the fibre by the electron irradiation. However, the pattern is distorted by the tilt angle of the viewing screen. A camera length of at least 2 m should be used when the ED pattern is recorded, if accurate measurement of the pattern is to be possible. It is necessary that, when obtaining an ED pattern to be evaluated visually or to be recorded, the sample height shall be properly adjusted to the eucentric point and the image shall be focused in the plane of the selected area aperture. If this is not done there may be some components of the ED pattern which do not originate from the selected area. In general, it will be necessary to use the smallest available ED aperture.

For accurate measurements of the ED pattern, an internal calibration standard shall be used. A thin coating of gold, or other suitable calibration material, shall be applied to the underside of the TEM specimen. This coating may be applied either by vacuum evaporation or, more conveniently, by sputtering. The polycrystalline gold film yields diffraction rings on every ED pattern and these rings provide the required calibration information.

To form an ED pattern, move the image of the fibre to the centre of the viewing screen, adjust the height of the specimen to the eucentric position, and insert a suitable selected area aperture into the electron beam so that the fibre, or a portion of it, occupies a large proportion of the illuminated area. The size of the aperture and the portion of the fibre shall be such that particles other than the one to be examined are excluded from the selected area. Observe the ED pattern through the binoculars. During the observation, the objective lens current should be adjusted to the point where the most complete ED pattern is obtained. If an incomplete ED pattern is still obtained, move the particle around within the selected area to attempt to optimize the ED pattern, or to eliminate possible interferences from neighbouring particles.

If a zone-axis ED analysis is to be attempted on the fibre, the sample shall be mounted in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fibre image indicates that the fibre is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fibre is at the eucentric position. Tilt the fibre until an ED appears which is a symmetrical, two-dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. During tilting of the fibre to obtain zone-axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections occur at some points on a matrix of strong reflections, the possibility of twinning or multiple diffraction exists, and some caution should be exercised in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the references by J.A. Gard<sup>[20]</sup>, P.B. Hirsch, et al.<sup>[22]</sup>, and H.R. Wenk<sup>[36]</sup>, included in the Bibliography. Not all zone-axis patterns which can be obtained are definitive. Only those which have closely spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0,3 nm are not definitive. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are the most definitive.

Five spots, closest to the centre spot, along two intersecting lines of the zone-axis pattern shall be selected for measurement, as illustrated in [Figure D.1](#). The distances of these spots from the centre spot and the four angles shown provide the required data for analysis. Since the centre spot is usually very over-exposed, it does not provide a well-defined origin for these measurements. The required distances shall therefore be obtained by measuring between pairs of spots symmetrically disposed about the centre spot, preferably separated by several repeat distances. The distances shall be measured with a precision of better than 0,3 mm, and the angles to a precision of better than 2,5°. The diameter, D, of the first or second ring of the calibration pattern (111 and 200) shall also be measured with a precision of better than 0,3 mm.

Using gold as the calibration material, the radius-based camera constant is given by:

- $\lambda \cdot L = 0,117\ 74 \times D\ \text{mm} \cdot \text{nm}$  (first ring)
- $\lambda \cdot L = 0,101\ 97 \times D\ \text{mm} \cdot \text{nm}$  (second ring)

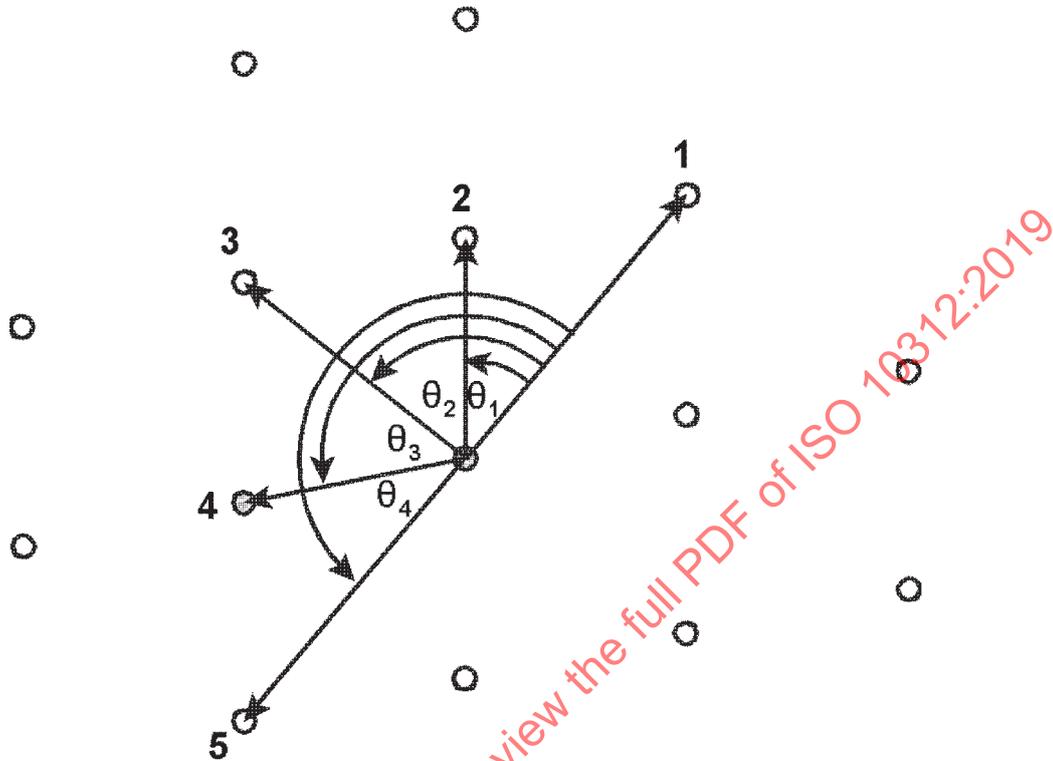


Figure D.1 — Example of measurement of zone-axis SAED patterns

### D.2.3 EDXA measurements

Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the X-ray peaks originating from the elements in the fibre are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the X-ray peaks originating from the elements in the fibre. This method provides quantitative interpretation for those minerals which contain silicon.

To obtain an EDXA spectrum, move the image of the fibre to the centre of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fibre. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector and, in some instruments, to use Scanning Transmission Electron Microscopy (STEM) mode of operation.

The time for acquisition of a suitable spectrum varies with the fibre diameter, and also with instrumental factors. For quantitative interpretation, spectra should have a statistically valid number of counts in each peak. Analyses of small diameter fibres which contain sodium are the most critical, since it is in the low energy range that the X-ray detector is least sensitive. Accordingly, it is necessary to acquire a spectrum for a sufficiently long period that the presence of sodium can be detected in such fibres. It has been found that satisfactory quantitative analyses can be obtained if acquisition is continued until the background subtracted silicon  $K\alpha$  peak integral exceeds 10 000 counts. The spectrum should then be manipulated to subtract the background and to obtain the net areas of the elemental peaks.

After quantitative EDXA classification of some fibres by computer analysis of the net peak areas, it may be possible to classify further fibres in the same sample on the basis of comparisons of spectra at the instrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

## D.3 Interpretation of fibre analysis data

### D.3.1 Chrysotile

The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few other minerals have similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the pattern correspond to those from reference chrysotile. However, depending on the past history of the fibre, and on a number of other factors, the crystal structure of a particular fibre may be damaged, and it may not yield an ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

### D.3.2 Amphiboles

Since the fibre identification procedure for asbestos fibres other than chrysotile can be involved and time-consuming, computer programmes such as that developed by Rhoades<sup>[30]</sup> are recommended for interpretation of zone-axis ED patterns. The published literature<sup>[9][19]</sup> contains composition and crystallographic data for all the fibrous minerals likely to be encountered in TEM analysis of air samples, and the compositional and structural data from the unknown fibre should be compared with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fibre identified by quantitative EDXA and two low index zone axis ED patterns.

Suspected amphibole fibres should be classified initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, a list of minerals which are consistent in composition with that measured for the unknown fibre should be compiled. To proceed further, it is necessary to obtain the first zone axis ED pattern, according to the instructions in [D.2.2](#).

It is possible to specify a particular zone-axis pattern for identification of amphibole, since a few patterns are often considered to be characteristic. Unfortunately, for a fibre with random orientation on a TEM grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two pre-selected zone-axes. The most practical approach has been adopted, which is to accept those low index patterns which are easily obtained, and then to test their consistency with the structures of the minerals already pre-selected on the basis of the EDXA data. Even the structures of non-amphibole minerals in this pre-selected list shall be tested against the zone-axis data obtained for the unknown fibre, since non-amphibole minerals in some orientations may yield similar patterns consistent with amphibole structures.

The zone-axis ED interpretation shall include all minerals previously selected from the mineral data file as being chemically compatible with the EDXA data. This procedure will usually shorten the list of minerals for which solutions have been found. A second set of zone-axis data from another pattern obtained on the same fibre can then be processed, either as further confirmation of the identification, or to attempt elimination of an ambiguity. In addition, the angle measured between the orientations of the two zone-axes can be checked for consistency with the structures of the minerals. Caution should be exercised in rationalizing the inter-zone axis angle, since if the fibre contains *c*-axis twinning the two zone-axis ED patterns may originate from the separate twin crystals. In practice, the full identification procedure will normally be applied to very few fibres, unless for a particular reason precise identification of all fibres is required.

Use the International Mineralogical Association (IMA)<sup>[24][25]</sup> procedure for the nomenclature of amphiboles.

## D.4 Fibre classification categories

### D.4.1 General

It is not always possible to proceed to a definitive identification of a fibre; this may be due to instrumental limitations or to the actual nature of the fibre. In many analyses a definitive identification of each fibre may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure shall therefore take account of both instrumental limitations and varied analytical requirements. Accordingly, a system for fibre classification is used to permit accurate recording of data. The classifications are shown in [Tables D.1](#) and [D.2](#), and are directed towards identification of chrysotile and amphibole respectively. Fibres shall be reported in these categories. A situation may occur in which a structure incorporates both chrysotile and an amphibole, or more than one type of amphibole. This is usually a rare event. When this does occur, classify the primary structure as “Mixed”, using the code “M” instead of “C” or “A”.

The general principle to be followed in this analytical procedure is first to define the most specific fibre classification which is to be attempted, or the “level” of analysis to be conducted. Then, for each fibre examined, record the classification which is actually achieved. Depending on the intended use of the results, criteria for acceptance of fibres as “identified” can then be established at any time after completion of the analysis.

In an unknown sample, chrysotile will be regarded as confirmed only if a recorded, calibrated ED pattern from one fibre in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Amphibole will be regarded as confirmed only by obtaining recorded data which yields exclusively amphibole solutions for fibres classified in the AZQ, AZZ or AZZQ categories.

**Table D.1 — Classification of fibres with tubular morphology**

Category	Description
TM	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	Characteristic Chrysotile Morphology
CD	Chrysotile SAED pattern
CQ	Chrysotile composition by Quantitative EDXA
CMQ	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	Non-Asbestos Mineral

**Table D.2 — Classification of fibres without tubular morphology**

Category	Description
UF	Unidentified Fibre
AD	Amphibole by random orientation SAED (shows layer pattern of 0,53 nm spacing)
AX	Amphibole by qualitative EDXA. Spectrum has elemental composition consistent with amphibole
ADX	Amphibole by random orientation SAED and qualitative EDXA
AQ	Amphibole by Quantitative EDXA
AZ	Amphibole by one Zone-axis SAED pattern
ADQ	Amphibole by random orientation SAED and Quantitative EDXA
AZQ	Amphibole by one Zone-axis SAED pattern and Quantitative EDXA
AZZ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle
AZZQ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle, and Quantitative EDXA
NAM	Non-Asbestos Mineral

#### D.4.2 Procedure for classification of fibres with tubular morphology suspected to be chrysotile

Occasionally, fibres are encountered which have tubular morphology similar to that of chrysotile, but which cannot be characterized further either by ED or EDXA. They may be non-crystalline, in which case ED techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fibre may be of organic origin, but the morphology and composition may not be sufficiently definitive that it can be disregarded. Accordingly, there is a requirement to record each fibre, and to specify how confidently each fibre can be identified. Classification of fibres will meet with various degrees of success. [Figure D.2](#) shows the classification procedure to be used for fibres which display any tubular morphology. The chart is self-explanatory, and every fibre is either rejected as a non-asbestos mineral (NAM), or classified in some way which by some later criterion could still contribute to the chrysotile fibre count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fibre by ED and EDXA methods according to [Figure D.2](#). Where the morphology is more definitive, it may be possible to classify the fibre as having chrysotile morphology (CM).

For classification as CM, the morphological characteristics required are:

- a) the individual fibrils should have high aspect ratios exceeding 5:1, and be about 30 nm to 40 nm in diameter;
- b) the electron scattering power of the fibre at 60 kV to 100 kV accelerating potential should be sufficiently low for internal structure to be visible;
- c) there should be some evidence of internal structure suggesting a tubular appearance similar to that shown by reference UICC chrysotile, which may degrade in the electron beam.

Examine every fibre having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those which give diffraction patterns with the precise characteristics shown in [Figure D.3](#). Since chrysotile has cylindrical symmetry<sup>[38]</sup>, the ED pattern remains unchanged during

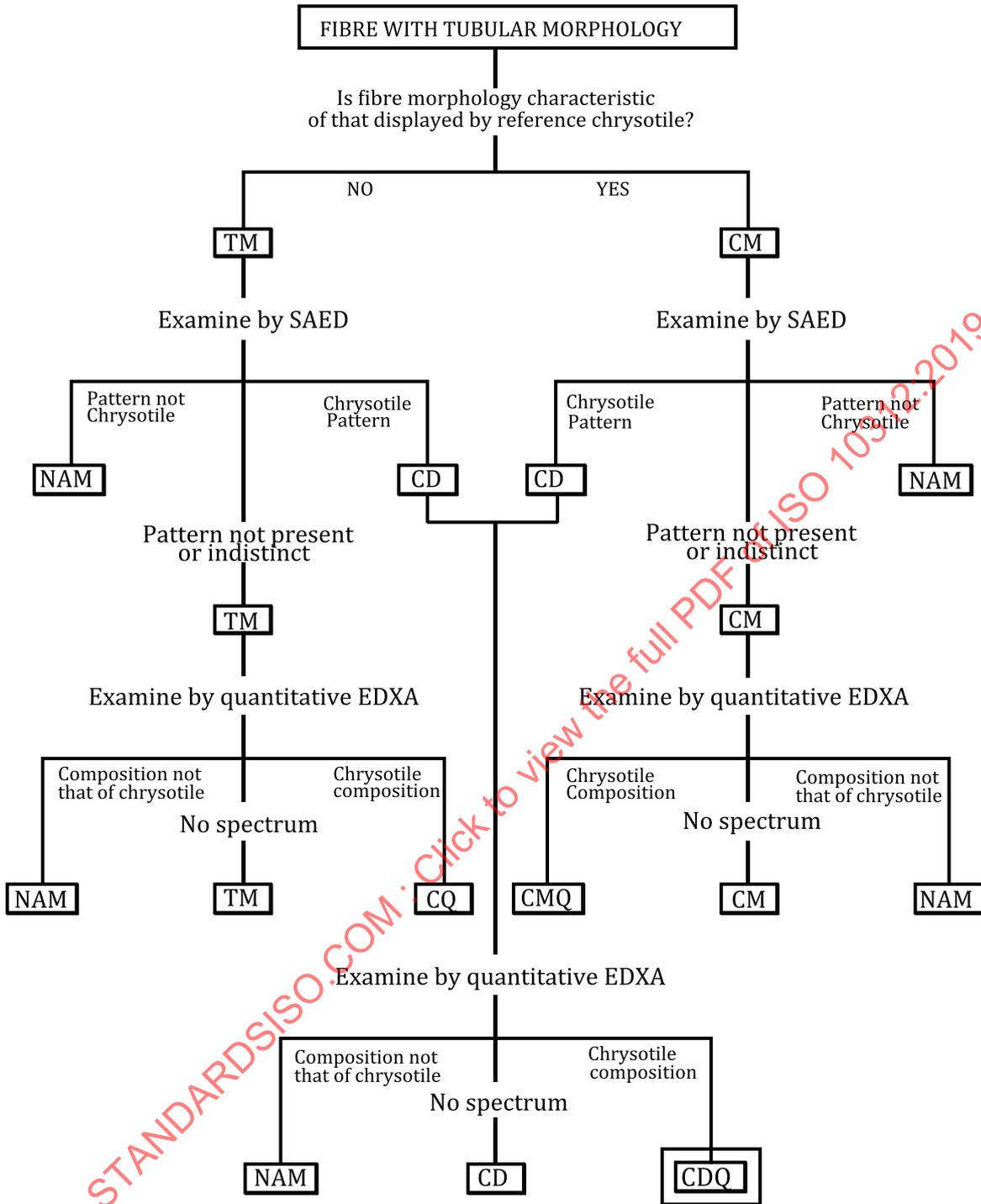


Figure D.2 — Classification chart for fibre with tubular morphology

axial rotation of the fibre. The relevant features in this pattern for identification of chrysotile are as follows:

- the (002) reflections should be examined to determine that they correspond closely to a spacing of 0,73 nm;
- the layer line repeat distance should correspond to 0,53 nm; and,
- there should be “streaking” of the (110) and (130) reflections.

Using the millimetre calibrations on the TEM viewing screen, these observations can readily be made at the instrument. If documentary proof of fibre identification is required, record a TEM micrograph of at least one representative fibre, and record its ED pattern on a separate film or plate. This film or plate shall also carry calibration rings from a known polycrystalline substance such as gold. This calibrated pattern is the only documentary proof that the particular fibre is chrysotile, and not some other tubular or scrolled species such as halloysite, palygorskite, talc, vermiculite<sup>[17]</sup> or lizardite<sup>[37]</sup>. The proportion of fibres which can be successfully identified as chrysotile by ED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibres that fail to yield an identifiable ED pattern will remain in the TM or CM categories unless they are examined by EDXA.

In the EDXA analysis of chrysotile there are only two elements which are relevant. For fibre classification, the EDXA analysis must be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their areas in the appropriate ratio, and with only minor peaks from other elements, classify the fibre as chrysotile by quantitative EDXA, in the categories CQ, CMQ, or CDQ, as appropriate.

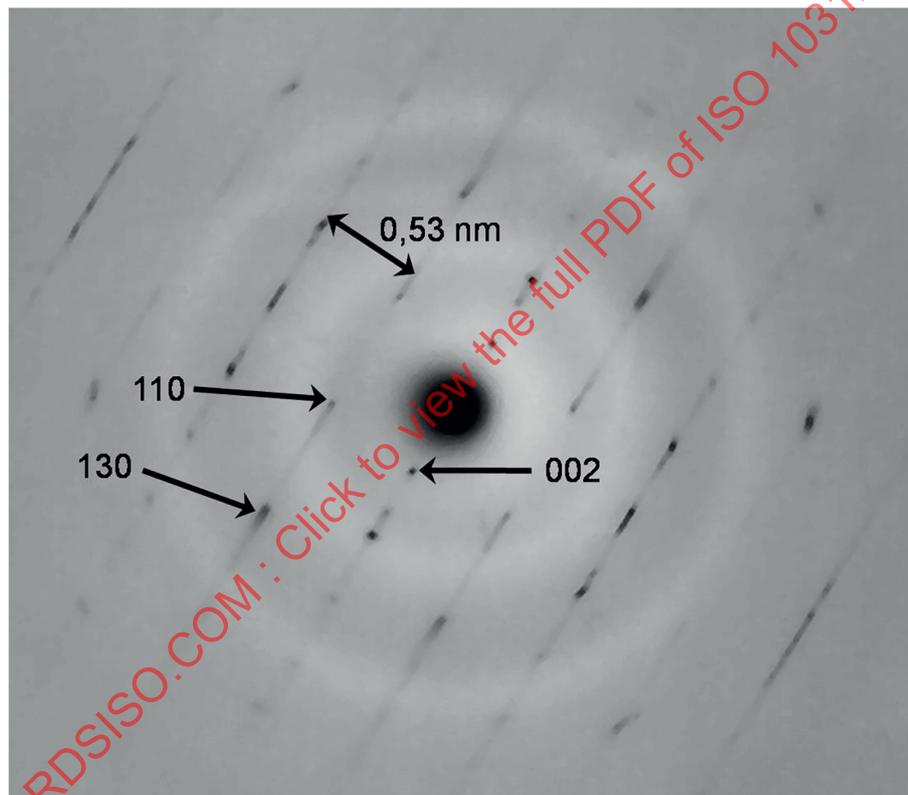


Figure D.3 — Chrysotile SAED pattern

#### D.4.3 Procedure for classification of fibres without tubular morphology, suspected to be amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 5:1 or greater, and having parallel or stepped sides, shall be considered as a suspected amphibole fibre. Further examination of the fibre by ED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fibre and on a number of instrumental limitations. It will not be possible to identify every fibre completely, even if time and cost are of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone-axis ED patterns, a very time-consuming procedure. Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone-axis ED work to a minimum of one fibre representative of each compositional class reported. In some samples, it may be necessary to identify more fibres by the zone-axis technique. When analysing samples from well-characterized sources, the cost of identification by zone-axis methods may not be justified.

The 0,53 nm layer spacing of the random orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of c-axis twinning in many fibres leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fibre aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fibre is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fibre may be some other mineral species.

[Figure D.4](#) shows the fibre classification chart to be used for suspected amphibole fibres. This chart shows all the classification paths possible in analysis of a suspected amphibole fibre, when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fibre by random orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fibre classification assigned will be defined either by successful analysis at the maximum required level, or by the instrumental limitations. Any instrumental limitations which affect the quality of the results shall be noted. Record the maximum classification achieved for each fibre on the counting sheet in the appropriate column. The various classification categories can then be combined later in any desired way for calculation of the fibre concentration. The complete record of the results obtained when attempting to identify each fibre can also be used to re-assess the data if necessary.

In the unknown sample, zone-axis analysis will be required if the presence of amphibole is to be unequivocally confirmed. For this level of analysis, attempt to raise the classification of every suspected amphibole fibre to the ADQ category by inspection of the random orientation ED pattern and the EDXA spectrum. In addition, examine at least one fibre from each type of suspected amphibole found by zone-axis methods to confirm their identification. In most cases, because information exists about possible sources of asbestos in close proximity to the air sampling location, some degree of ambiguity of identification can be accepted. Lower levels of analysis can therefore be accepted for these situations.

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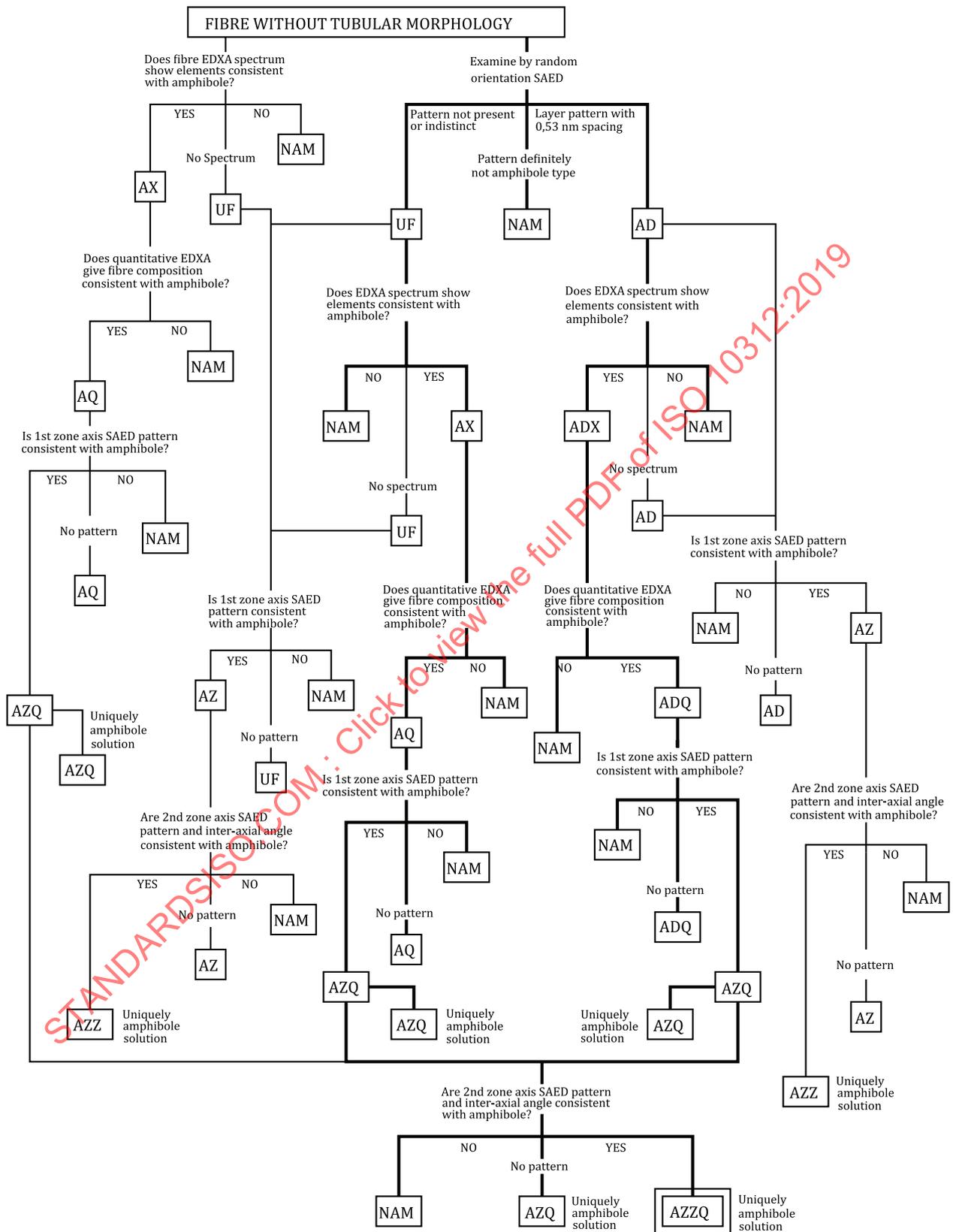


Figure D.4 — Classification chart for fibre without tubular morphology

## D.5 Routine fibre identification for investigation of known sources of the regulated asbestos varieties

For analyses in which the minerals of interest are limited to investigations of known sources of the regulated commercial asbestos varieties or richterite-winchite, a simpler procedure for fibre identification may be used. The compositions of the major three commercial regulated asbestos varieties (chrysotile, amosite and crocidolite) exhibit only limited variation with respect to the major chemical elements (sodium, magnesium, silicon and iron). Amosite may or may not exhibit a small EDXA peak from manganese.

For chrysotile, observation of the characteristic tubular morphology, combined with an EDXA spectrum closely similar to that of reference chrysotile, is sufficient for identification. For amosite and crocidolite, a layer type random orientation ED pattern, as specified in [D.4.3](#), from at least one typical fibre, combined with an EDXA spectrum closely similar to that of the reference amphibole asbestos, is sufficient.

Tremolite, actinolite, anthophyllite and richterite-winchite vary in composition. If a known source is under investigation, a layer type random orientation ED pattern from at least one typical fibre, combined with EDXA spectra that are closely similar to those obtained from the known source material is permissible as sufficient identification. If the source is not known, identification shall be performed using the standard procedures of this Annex.

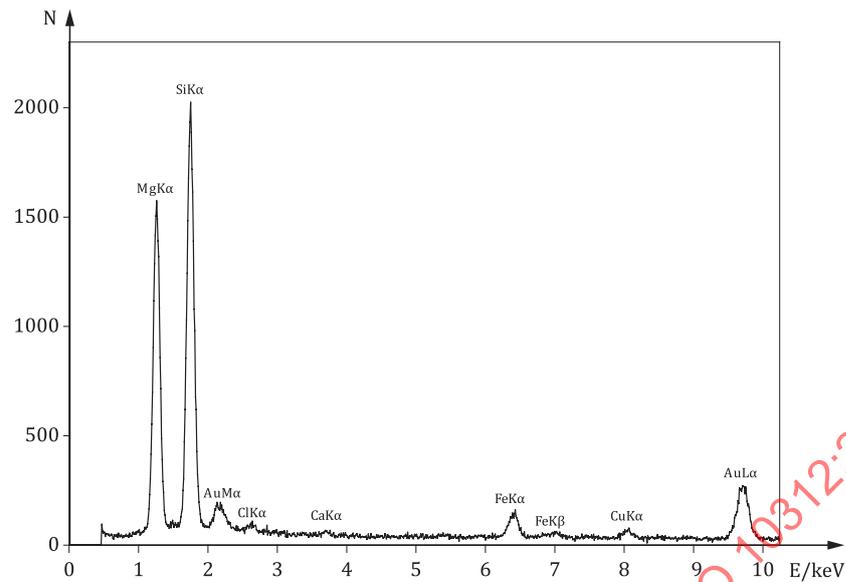
The reference EDXA spectra that follow are shown as examples only. They were acquired from TEM specimens prepared on gold grids, using an X-ray detector with a beryllium window. X-ray detectors with ultra-thin windows produce EDXA spectra with higher efficiency for elements of low atomic number. It is necessary to generate reference EDXA spectra specifically for the system in use. If field samples are prepared on copper TEM grids, detectability of the sodium  $K\alpha$  peak of crocidolite should be demonstrated by use of crocidolite fibres prepared on a copper TEM grid.

Reference EDXA spectra for NIST SRM 1866 chrysotile, amosite and crocidolite are shown in [Figures D.5](#) to [D.7](#). Although crocidolite characterized by a significantly higher concentration of magnesium and a lower concentration of iron than other sources of crocidolite is unlikely to be encountered, an EDXA spectrum for a Bolivian crocidolite with these characteristics is shown in [Figure D.8](#).

[Figure D.9](#) shows an EDXA spectrum from NIST SRM 1867 anthophyllite, and [Figure D.10](#) shows an EDXA spectrum from HSE Reference anthophyllite. These two sources of anthophyllite have different iron concentrations, and anthophyllite may be encountered that has a significantly lower iron concentration than those shown in [Figures D.9](#) and [D.10](#). In some sources of anthophyllite, iron is not detectable by EDXA.

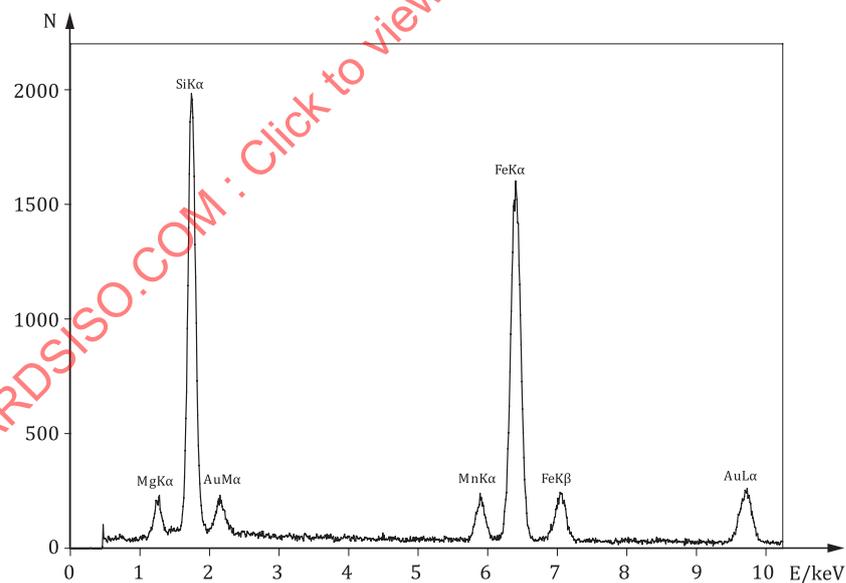
[Figures D.11](#) and [D.12](#) show EDXA spectra obtained from NIST SRM 1867 tremolite asbestos and NIST SRM 1867 actinolite asbestos. NIST SRM 1867 tremolite asbestos has an iron concentration just below the boundary between tremolite and actinolite defined by the International Mineralogical Association. NIST SRM 1867 actinolite asbestos has an iron concentration just above this boundary. Accordingly, these two reference samples are useful for classification of unknown tremolite-actinolite fibres. The EDXA spectrum from HSE reference tremolite, shown in [Figure D.13](#), is an example of tremolite with a lower iron concentration. The EDXA spectrum from HSE reference actinolite, shown in [Figure D.14](#), is an example of actinolite with a high iron concentration.

[Figure D.15](#) shows an EDXA spectrum obtained from richterite-winchite. This mineral is associated with some sources of vermiculite. [Figure D.15](#) should be considered only as an example, because the concentrations of sodium, potassium and calcium can be quite variable, even within materials from the same source.

**Key** $N$  counts $E$  X-ray energy

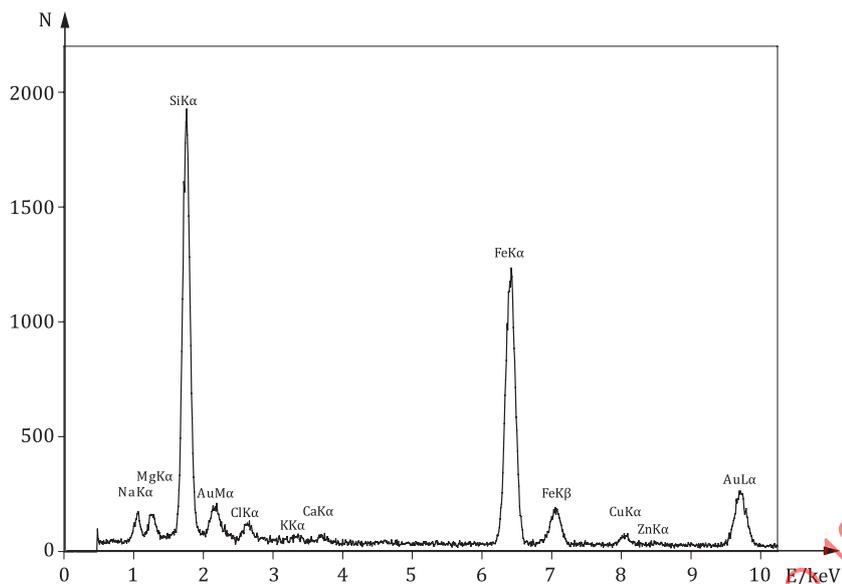
NOTE The gold and small copper peaks originate from the gold specimen grid.

**Figure D.5 — Energy dispersive X-ray spectrum obtained from SRM 1866 chrysotile**

**Key** $N$  counts $E$  X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

**Figure D.6 — Energy dispersive X-ray spectrum obtained from SRM 1866 amosite**



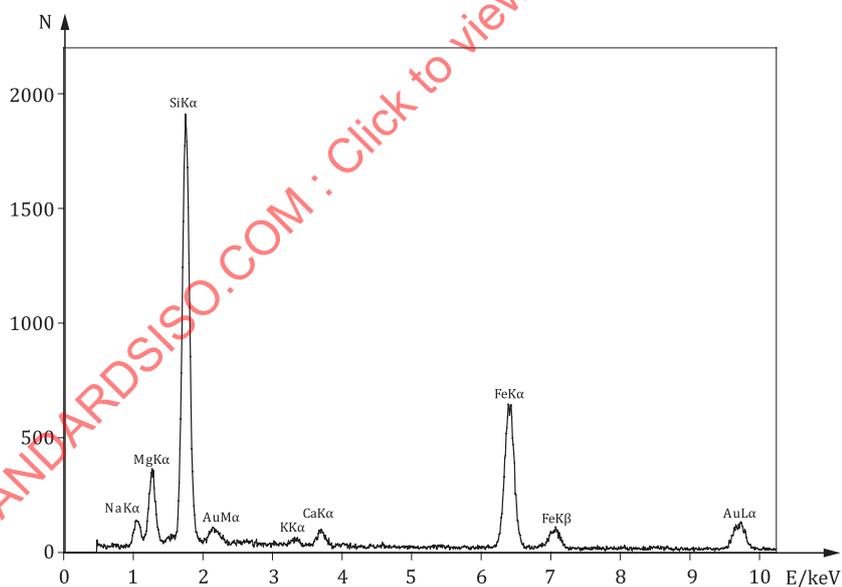
**Key**

$N$  counts

$E$  X-ray energy

NOTE The gold and small copper peaks originate from the gold specimen grid.

**Figure D.7 — Energy dispersive X-ray spectrum obtained from SRM 1866 crocidolite**



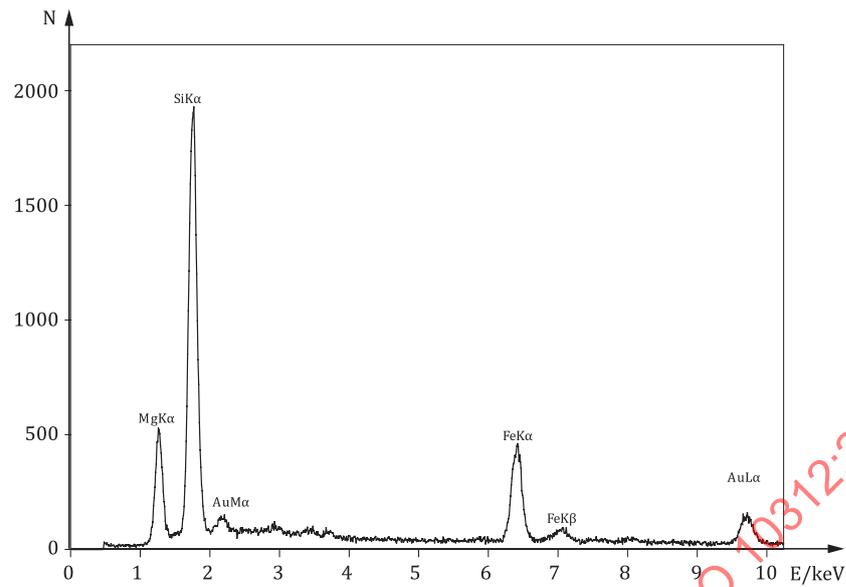
**Key**

$N$  counts

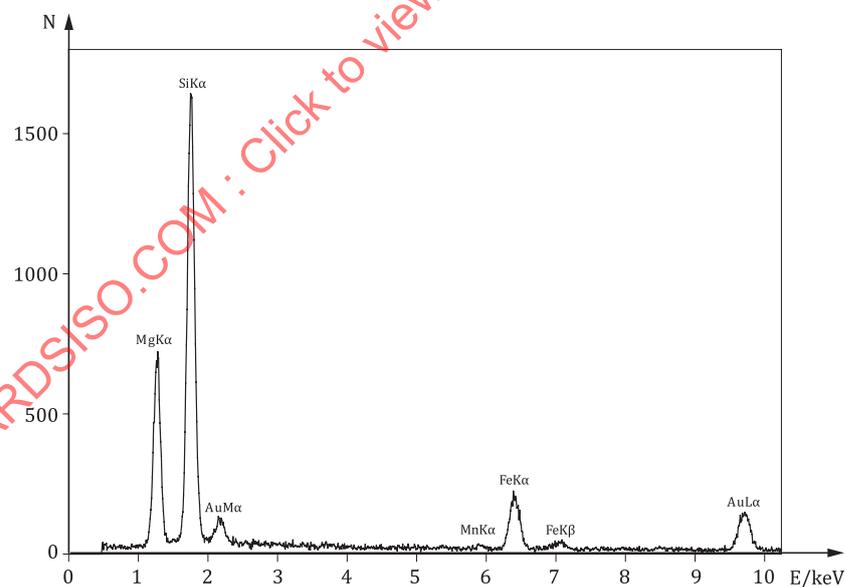
$E$  X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

**Figure D.8 — Energy dispersive X-ray spectrum obtained from Bolivian crocidolite**

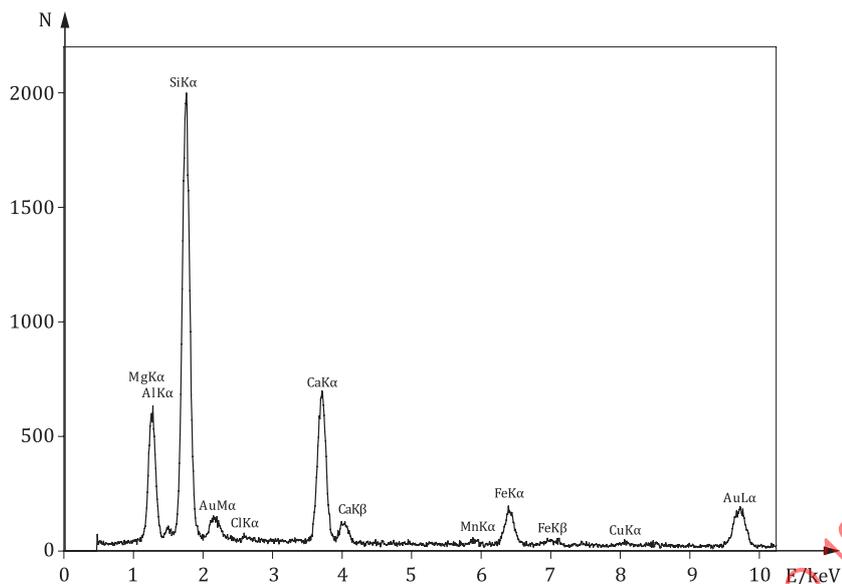
**Key** $N$  counts $E$  X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

**Figure D.9 — Energy dispersive X-ray spectrum obtained from SRM 1867 anthophyllite****Key** $N$  counts $E$  X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

**Figure D.10 — Energy dispersive X-ray spectrum obtained from HSE anthophyllite**



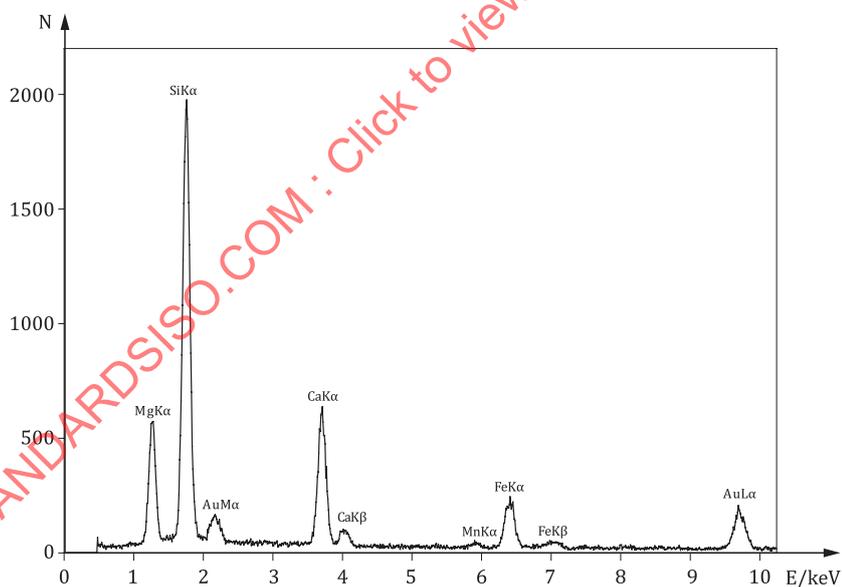
**Key**

$N$  counts

$E$  X-ray energy

NOTE The gold and small copper peaks originate from the gold specimen grid.

**Figure D.11 — Energy dispersive X-ray spectrum obtained from SRM 1867 tremolite**



**Key**

$N$  counts

$E$  X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

**Figure D.12 — Energy dispersive X-ray spectrum obtained from SRM 1867 actinolite**