INTERNATIONAL STANDARD

ISO 13794

Second edition
2019-10

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

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

Reference number
ISO 13794:2019(E)

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Published in Switzerland

Contents

Page

Foreword

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

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

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

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

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

Introduction

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

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

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

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

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

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

1 Scope

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

2 Normative references

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

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

3 Terms and definitions

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
acicular
shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like

[SOURCE: ISO 10312:1995, 3.1]

3.2
amphibole
group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:

$$A_{0\text{-}1}B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where

A is K, Na;

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

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

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

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

[SOURCE: ISO 10312:1995, 3.2]

3.3
amphibole asbestos
amphibole in an asbestiform habit

[SOURCE: ISO 10312:1995, 3.3]

3.4
analytical filter
filter through which an aqueous dispersion of ash from the sample collection filter is passed, and from which transmission electron microscope specimen grids are prepared

[SOURCE: ISO 13794:1999, 2.4]

3.5
analytical sensitivity
calculated airborne asbestos structure concentration in structures/litre, equivalent to counting of one asbestos structure in the analysis

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

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

[SOURCE: ISO 10312:1995, 3.4]

3.6
asbestiform
specific type of mineral fibrosity in which the fibres and fibrils possess high tensile strength and flexibility

[SOURCE: ISO 10312:1995, 3.5]

3.7
asbestos
group of silicate minerals belonging to the serpentine and amphibole groups which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong fibres when crushed or processed

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

[SOURCE: ISO 10312:1995, 3.6]

3.8

asbestos structure

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

[SOURCE: ISO 10312:1995, 3.7]

3.9

ashed filter blank

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

[SOURCE: ISO 13794:1999, 2.9]

3.10

aspect ratio

ratio of length to width of a particle

[SOURCE: ISO 10312:1995, 3.8]

3.11

blank

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

[SOURCE: ISO 10312:1995, 3.9]

3.12

camera length

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

[SOURCE: ISO 10312:1995, 3.10]

3.13

chrysotile

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

$$Mg_3Si_2O_5(OH)_4$$

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.

[SOURCE: ISO 10312:1995, 3.11]

3.14

cleavage

breaking of a mineral along one of its crystallographic directions

[SOURCE: ISO 10312:1995, 3.12]

3.15

cleavage fragment

fragment of a crystal that is bounded by cleavage faces

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

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

3.16
cluster
structure in which two or more fibres, or fibre bundles, are randomly oriented in a connected grouping

[SOURCE: ISO 10312:1995, 3.14]

3.17
direct-transfer blank
structure count made on transmission electron microscope specimens prepared by the direct-transfer procedure from a blank filter of the type used for filtration of aqueous dispersions of ash

[SOURCE: ISO 13794:1999, 2.17]

3.18
d-spacing
distance between identical adjacent and parallel planes of atoms in a crystal

[SOURCE: ISO 10312:1995, 3.15]

3.19
electron diffraction
technique in electron microscopy by which the crystal structure of a specimen is examined

[SOURCE: ISO 10312:1995, 3.16]

3.20
electron scattering power
extent to which a thin layer of substance scatters electrons from their original directions

[SOURCE: ISO 10312:1995, 3.17]

3.21
empty beaker blank
fibre count made on transmission electron microscope specimens prepared by the indirect procedure using an empty beaker as the initial sample

[SOURCE: ISO 13794:1999, 2.21]

3.22
energy dispersive X-ray analysis
measurement of the energies and intensities of X-rays by use of a solid state detector and multi-channel analyser system

[SOURCE: ISO 10312:1995, 3.18]

3.23
eucentric
condition when the area of interest of an object is placed on a tilting axis at the intersection of the electron beam with that axis and is in the plane of focus

[SOURCE: ISO 10312:1995, 3.19]

3.24
field blank
filter cassette that has been taken to the sampling site, opened and then closed and which is used to determine the background structure count for the measurement

[SOURCE: ISO 10312:1995, 3.20]

3.25
fibril
single fibre of asbestos, which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances

[SOURCE: ISO 10312:1995, 3.21]

3.26
fibre
elongated particle which has parallel or stepped sides

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

[SOURCE: ISO 10312:1995, 3.22]

3.27
fibre bundle
structure composed of parallel, smaller diameter fibres attached along their lengths

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

[SOURCE: ISO 10312:1995, 3.23]

3.28
fibrous structure
fibre, or connected grouping of fibres, with or without other particles

[SOURCE: ISO 10312:1995, 3.24]

3.29
funnel blank
structure count made on transmission electron microscope specimens prepared by the direct-transfer method from a filter used for filtration of a sample of distilled water

[SOURCE: ISO 13794:1999, 2.29]

3.30
habit
characteristic crystal growth form or combination of these forms of a mineral, including characteristic irregularities

[SOURCE: ISO 10312:1995, 3.25]

3.31
limit of detection
calculated airborne fibre concentration in structures/l, equivalent to the upper 95 % confidence limit of 2,99 structures predicted by the Poisson distribution for a count of zero structures

[SOURCE: ISO 10312:1995, 3.26]

3.32
matrix
structure in which one or more fibres, or fibre bundles, touch, are attached to, or partially concealed by, a single particle or connected group of non-fibrous particles

[SOURCE: ISO 10312:1995, 3.27]

3.33
Miller index

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

[SOURCE: ISO 10312:1995, 3.28]

3.34
PCM equivalent fibre

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

[SOURCE: ISO 10312:1995, 3.29]

3.35
PCM equivalent structure

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

[SOURCE: ISO 10312:1995, 3.30]

3.36
pixel

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

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

3.37
primary structure

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

[SOURCE: ISO 10312:1995, 3.31]

3.38
replication

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

[SOURCE: ISO 10312:1995, 3.32]

3.39
selected area electron diffraction

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

[SOURCE: ISO 10312:1995, 3.33]

3.40
serpentine

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

$$Mg_3Si_2O_5(OH)_4$$

[SOURCE: ISO 10312:1995, 3.34]

3.41
structure

single fibre, fibre bundle, cluster or matrix

[SOURCE: ISO 10312:1995, 3.35]

3.42
twinning
occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law

[SOURCE: ISO 10312:1995, 3.36]

3.43
unopened fibre
large diameter asbestos fibre bundle that has not been separated into its constituent fibrils or fibres

[SOURCE: ISO 10312:1995, 3.37]

3.44
zone-axis
line or crystallographic direction through the centre of a crystal that is parallel to the intersection edges of the crystal faces defining the crystal zone

[SOURCE: ISO 10312:1995, 3.38]

4 Symbols and abbreviated terms

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

5 Type of sample

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

6 Range

The upper limit for the range of concentration that can be measured on the analytical filter is 7 000 structures/mm^2. The lower limit of the range that can be measured on the analytical filter corresponds to the detection of 2,99 structures in the area of specimen examined. Measurement of concentrations lower than 1 structure/mm^2 can be achieved. The air concentrations represented by these values are a function of the volume of air sampled and the degree of dilution or concentration selected during the specimen preparation procedures. The method is particularly applicable to measurements in areas with high suspended particulate concentrations (exceeding 25 µg/m^3), or where detection and identification of asbestos fibres are likely to be prevented or hindered by other types of particulate in direct-transfer TEM preparations. In theory, there is no lower limit to the dimensions of asbestos fibres that can be detected. In practice, microscopists vary in their ability to detect very short asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

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

7 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air, concentrating the sample during specimen preparation, and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of an examined TEM specimen is controlled by the total suspended particulate concentration remaining after the ashing and aqueous dispersal steps, and this depends on the chemical nature of the suspended particulate. For total suspended particulate concentrations of approximately 10 µg/m^3, 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^2 of the TEM specimens is examined. For fibres longer than 5 µm, examined at lower magnifications, this limit of detection can be reduced by a further order of magnitude. Lower limits of detection can be achieved by increasing the area of the TEM specimens that is examined, or by concentration of the sample during specimen preparation. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre.

8 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a capillary-pore polycarbonate (PC) membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,8 µm by means of a battery-powered or mains-powered pump. A portion of the filter is ashed in an oxygen plasma[22], and the residual ash is dispersed in distilled water with adjustment of the pH to between 3,0 and 4,0 using acetic acid. Analytical filters are then prepared by filtration of known volumes of this aqueous dispersion through either capillary-pore polycarbonate membrane filters of maximum pore size 0,2 µm or cellulose ester membrane filters of maximum pore size 0,22 µm. TEM specimens are prepared from polycarbonate analytical filters by a carbon replication procedure[10] in which a thin film of carbon is applied to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film carbon replica which bridges the openings in the TEM specimen grid, and which supports each particle from the analytical filter in its original position. Cellulose ester analytical filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma[22] to ensure that all particles are exposed[11]. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

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

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

9 Reagents

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

9.1 Water, fibre-free.

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

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

9.3 1-Methyl-2-pyrrolidone.

9.4 1,2-Diaminoethane (Ethylene diamine).

9.5 Dimethylformamide.

9.6 Glacial acetic acid.

9.7 Acetone.

10 Apparatus

10.1 Air sampling

10.1.1 Filter cassette

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

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

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

10.1.2 Sampling pump

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

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

10.1.3 Stand

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

10.1.4 Personal sampling

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

10.1.5 Flowmeter

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

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

10.2 Specimen preparation laboratory

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

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

10.3 Equipment for analysis

10.3.1 Transmission electron microscope

A TEM[3] operating at an accelerating potential of 80 kV or greater, with a resolution better than 1,0 nm, and a magnification range of approximately × 300 to × 100 000 shall be used. The ability to obtain a viewing screen magnification of about × 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 ±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 µm^2 or less, selected from an in-focus image at a screen magnification of × 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):

$$A = 0{,}785\,4 \times \left(\frac{D}{M} + 2\,000 C_s \theta^3 \right)^2 \qquad (1)$$

where

A is the effective SAED area, in square micrometres;

D is the diameter, in micrometres, of the SAED aperture;

M is the magnification of the objective lens;

C_s is the spherical aberration coefficient, in millimetres, of the objective lens;

θ is the maximum required Bragg angle, in radians.

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

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

a) rotated through 360°, combined with tilting through at least +30° 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.

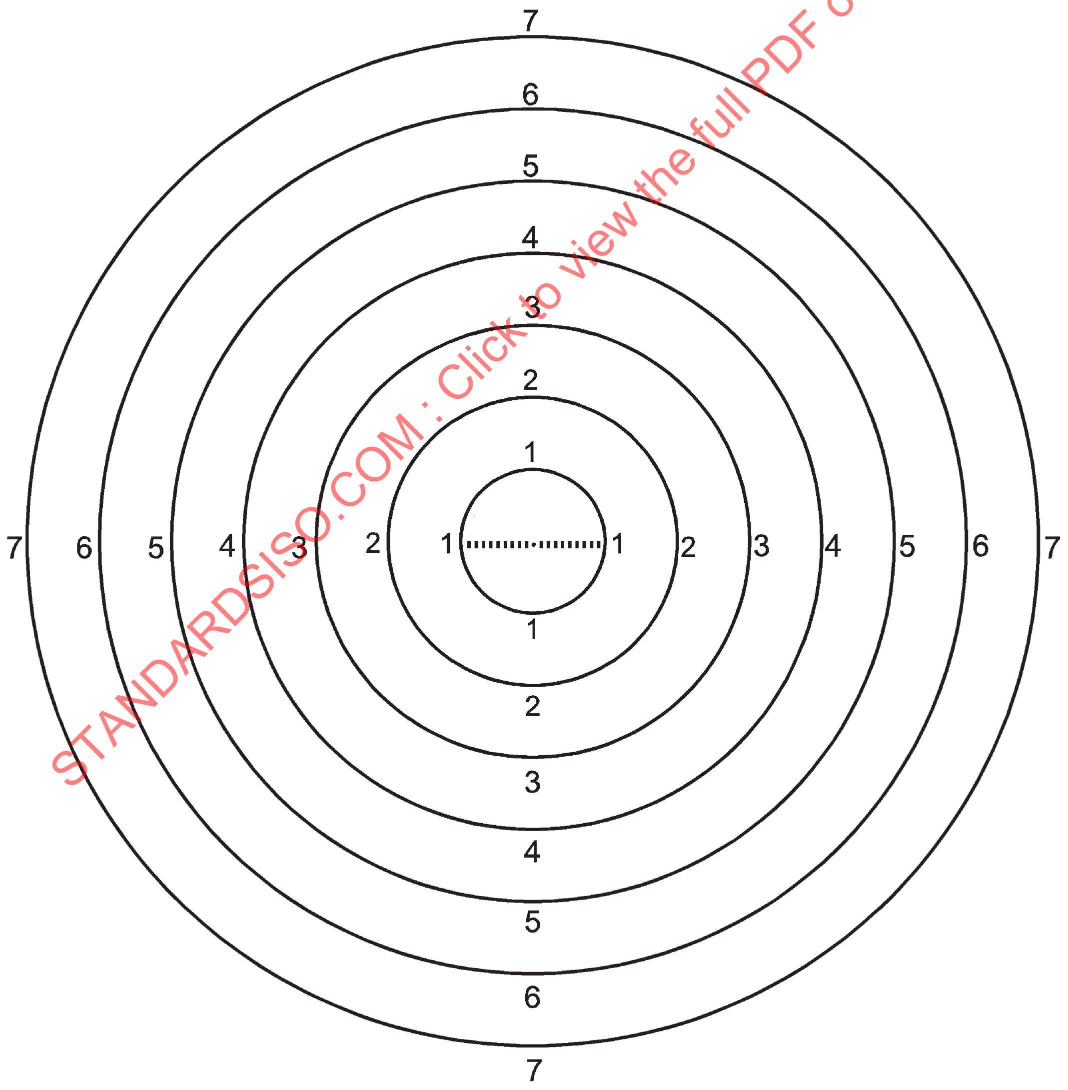


Figure 1 — Example of calibration markings on TEM viewing screen

10.3.2 Energy dispersive X-ray analyser

The TEM shall be equipped with an energy dispersive X-ray analyser capable of achieving a resolution better than 180 eV (FWHM) on the MnKα 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 NaKα integrated peak count rate of more than 1 count per second (cps) from a fibre of UICC crocidolite, 50 nm in diameter or smaller, when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1,0.

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

10.3.3 Plasma asher

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

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

10.3.4 Vacuum coating unit

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

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

10.3.5 Sputter coater

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

10.3.6 Beakers

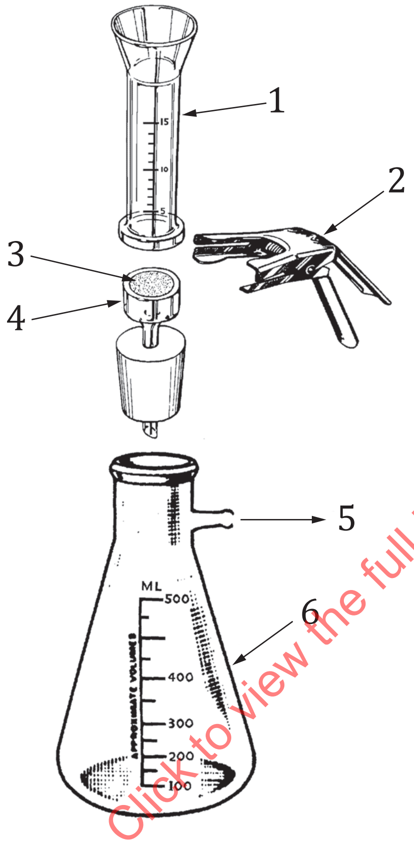
Borosilicate glass, 50 ml beakers are required as containers for plasma ashing of sample collection filters.

10.3.7 Vacuum source

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

10.3.8 Glass filtration apparatus

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



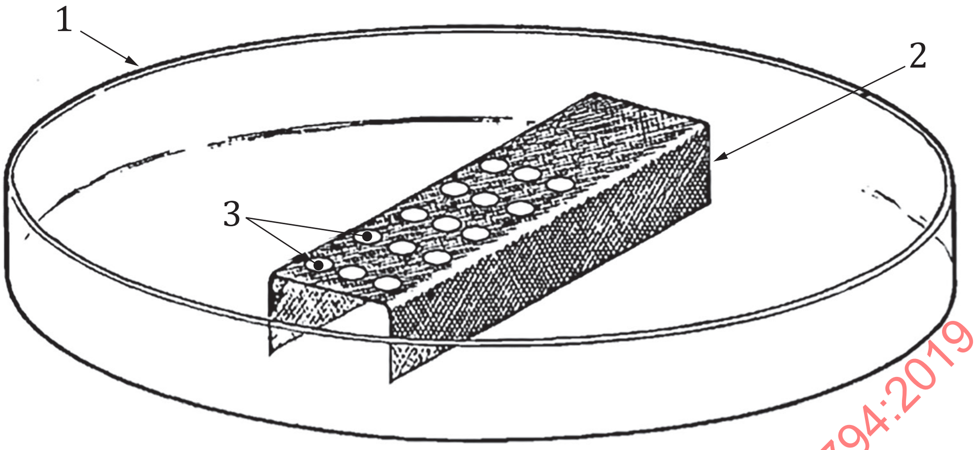
Key

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

Figure 2 — Example of filtration apparatus

10.3.9 Solvent washer (Jaffe washer)

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



Key

1 glass Petri dish (100 mm diameter, 15 mm deep)

2 stainless steel mesh bridge (50 mesh)

3 electron microscope specimen grids

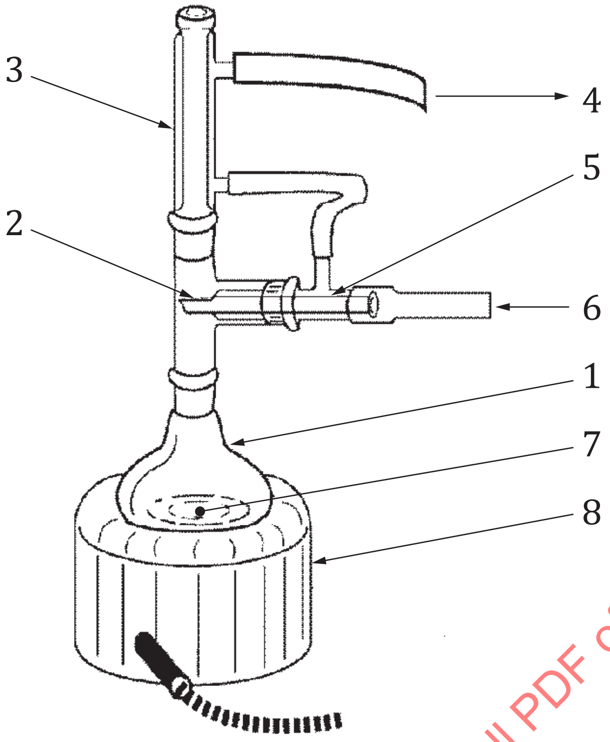
NOTE Solvent is added until the meniscus contacts the underside of the stainless-steel bridge.

Figure 3 — Example of Jaffe washer

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

10.3.10 Condensation washer

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



Key

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

Figure 4 — Design of condensation washer

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

10.3.11 Slide warmer or oven

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

10.3.12 Ultrasonic bath

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

10.3.13 Carbon grating replica

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

10.3.14 Calibration specimen grids for EDXA

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

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

10.3.15 Carbon rod sharpener

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

10.3.16 Disposable tip micropipettes

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

10.3.17 Thermometer

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

10.3.18 Stopwatch

A stopwatch is required for calibration of the ultrasonic bath.

10.4 Consumable supplies

10.4.1 Copper or nickel electron microscope grids

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

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

10.4.2 Gold or nickel electron microscope grids

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

10.4.3 Aluminium foil

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

10.4.4 Carbon rod electrodes

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

10.4.5 Routine electron microscopy tools and supplies.

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

10.4.6 Reference asbestos samples

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

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

10.4.7 Reference samples of mineral fibres other than asbestos

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

11 Air sample collection

11.1 Calculation of analytical sensitivity

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

$$S = \frac{\left(A_\mathrm{a} \cdot V_\mathrm{d}\right)}{\left(k \cdot A_\mathrm{g} \cdot V_\mathrm{f} \cdot F_\mathrm{a} \cdot V_\mathrm{s}\right)} \qquad (2)$$

where:

S the analytical sensitivity, expressed in structures/litre;

A_a the active area of the analytical filter in square millimetres;

V_d the volume, expressed in millilitres, of water used for dispersal of residual ash;

k the number of grid openings examined;

A_g the mean area of grid openings, expressed in square millimetres;

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

F_a the fraction of sample collection filter ashed;

V_s the volume of air sampled, in litres.

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

11.2 Sample collection procedure

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

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

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

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

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

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

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

12 Procedure for analysis

12.1 General

The procedures for ashing of sample collection filters, dispersion of the residual ash, and filtration of the aqueous dispersion are identical for the two types of sample collection filter. The techniques used to prepare TEM specimens from the analytical filters are different for polycarbonate and cellulose ester filters. The preparation method to be used shall be either 12.4 or 12.5, 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, use disposable supplies.

In order to ensure that all particulate contamination and fibres are detached from glassware, it is recommended that all surfaces be washed by rubbing vigorously with clean paper towel soaked in detergent, and rinsing several times under a continuous flow of water. The glassware should then be rinsed at least twice using fibre-free water.

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 with wet paper towel before it is taken into the clean facility or laminar flow hood.

12.3 Preparation of analytical filters

12.3.1 Selection of filter area for ashing

Depending on the analytical sensitivity to be achieved, ash either a quarter or a half of the sample collection filter. Using freshly-cleaned tweezers, remove the sample collection 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 required area of the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary.

12.3.2 Ashing of sample collection filters

Place the selected filter portion into a 50 ml borosilicate glass beaker, ensuring that the side of the filter with the collected particulate faces downwards. Cover the beaker with a 6 cm × 6 cm square piece of 0,015 mm to 0,02 mm thickness aluminium foil, and bend the foil over the edges of the beaker so that it forms a tight seal. Perforate the foil in 10 – 20 positions with a needle to allow for gas exchange during plasma ashing. Depending on the size of the plasma asher chamber, several sample filters may be ashed simultaneously. In order to provide a control to detect contamination of unused filters, it is recommended that an ashed filter blank be included with the samples. At least one empty beaker, prepared in the same way, shall be placed in the asher chamber along with each batch of samples ashed. Operate the plasma asher using the minimum power at which a glow discharge is observed, until the filters appear to be completely ashed. Increase the plasma asher power to the maximum, and operate under these conditions for a minimum period of 3 h. After the ashing treatment is completed, admit air to the chamber and remove the beakers.

Losses of particulate and fibres from the beaker will occur if the plasma asher is operated at excessive radio-frequency power. During ashing of MEC or cellulose nitrate filters, a critical point is reached during the oxidation at which a sudden, violent ignition occurs if the radio-frequency power is excessive. This results in loss of fibres from the beaker, contamination of the interior surfaces of the chamber, and possible cross-contamination of the samples. The same considerations apply to the ashing of PC filters. The ashing procedure specified avoids these problems. It is recommended that the ashing of a blank filter be observed closely during the final stages of oxidation, in order to ensure that the radio-frequency power setting is such that a sudden ignition does not occur.

NOTE 1 Operation of the plasma asher at excessive radio-frequency power during the initial stages of ashing can also result in shrinkage of the filter into a hard fragment of plastic that will not ash completely. If this occurs, the particulate material on the filter will not disperse in water during subsequent steps in the preparation procedure.

12.3.3 Aqueous dispersal of residual ash from sample collection filters

Remove the aluminium foil from the top of the beaker. Using a disposable tip pipette, add 50 µl of glacial acetic acid, and then add 40 ml of freshly-distilled water. Cover the beaker with a new 6 cm × 6 cm square piece of aluminium foil, and bend the foil over the edges of the beaker so that it forms a tight seal. Place the beaker into the ultrasonic bath and operate the bath at the calibrated power in accordance with Annex B for a period of 5 min.

12.3.4 Assembly of system for filtration of aqueous dispersions

Filtration of the aqueous dispersions is a very critical procedure because it is important to obtain uniform deposits of particulate on the analytical filters. The following procedure shall be used:

a) set up the filtration system and connect to a vacuum source;

b) add freshly distilled water to the filtration unit base component until there is a raised meniscus;

c) place a 5 µm pore size cellulose ester filter on to the water meniscus. The filter will centralize. Apply the vacuum very briefly in order to bring the filter into contact with the base component;

d) add freshly distilled water to the top of the cellulose ester filter, and place the analytical filter (either a 0,2 µm maximum pore size capillary-pore polycarbonate filter or a 0,22 µm maximum pore size cellulose ester filter) on to the water surface; apply the vacuum very briefly again in order to bring both filters into contact with the base component;

e) install the filtration reservoir and clamp the assembly together.

12.3.5 Filtration of aqueous dispersions

Before filtering the aqueous dispersions, prepare a funnel blank by filtration of 40 ml of freshly-distilled water.

The volume of the aqueous dispersion to be filtered depends on either the particulate concentration or the asbestos fibre concentration. The volume of the aqueous dispersion required to produce an analytical filter with a suitable particulate or fibre loading for analysis often cannot be predicted, and it is usually necessary to prepare several analytical filters corresponding to filtration of different aliquots. The aqueous dispersions are not stable, and extended exposure of chrysotile to acidic environments causes leaching of magnesium to occur from the fibres; it is therefore necessary to prepare all analytical filters immediately. If a proportion of the particulate material on the original sample filter is acid-soluble, add 2 ml of 0,1 M hydrochloric acid to the aqueous dispersion, and allow the reaction to proceed for 1 min. Uniform deposits of particulate on the analytical filters cannot be ensured if liquid volumes smaller than 5 ml are filtered using filtration systems of 199 mm^2 active area; accordingly, where it is required to filter volumes smaller than 5 ml, the aliquot shall be diluted with freshly-distilled water to a volume exceeding 5 ml.

Pour the aliquot of the dispersion into the filtration reservoir and apply the vacuum. If the volume of the aliquot is larger than the capacity of the filtration reservoir, do not allow the level of liquid in the reservoir to fall below 5 cm depth before the remaining volume is added. Failure to observe this precaution may result in disturbance of the filtered particulate and non-uniform deposition.

With the vacuum still applied, unclamp the filtration assembly and remove the filtration reservoir. Using clean tweezers, remove the analytical filter and transfer it to a petri-dish. Allow the filter to dry before placing the cover on the petri-dish.

For the beaker blank and any ashed filter blank, prepare only one analytical filter by filtration of the entire 40 ml dispersion.

It is recommended that analytical filters corresponding to volumes of 1 ml, 5 ml and 34 ml be prepared, unless there is reason to suspect that even the lowest volume will yield an analytical filter of unacceptably high loading. If the particulate or fibre concentration is thought to be such that it is required to filter an aliquot of lower volume than 1 ml, use a dilution procedure in which 1 ml of the original dispersion is transferred to a clean beaker and diluted with freshly-distilled water to a total volume of 100 ml. After stirring to ensure complete mixing, aliquots of 10 ml and 30 ml from this diluted dispersion can then be filtered, corresponding to volumes of 0,1 ml and 0,3 ml of the original dispersion. From the original dispersion, volumes of 1 ml, 5 ml and 33 ml are also filtered, giving 5 analytical filters with a concentration range of a factor of 330. The requirement for washing of the filtration apparatus is minimized if the aliquots are filtered in order of increasing concentration. If aqueous dispersions are known to have high concentrations of asbestos fibres, the level of cross-contamination from one sample to the next can be determined by interposing additional funnel blanks. Wash the filtration apparatus between filtrations of different samples.

12.4 Preparation of TEM specimens from PC analytical filters

12.4.1 Selection of filter area for carbon-coating

Use a cleaned microscope slide to support representative portions of PC filter during the carbon evaporation. Double-coated adhesive tape is used to hold the filter portions to the glass slide. Take care not to stretch the PC filters during handling. Using freshly-cleaned tweezers, remove the PC filter from the Petri-dish, 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. Include a portion of an unused PC filter as a direct-transfer blank. 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.

12.4.2 Carbon-coating of filter portions

Place the glass slide holding the filter portions on the rotation-tilting device, approximately 10 cm–12 cm from the evaporation source, and evacuate the evaporator chamber 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 chloroform, 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–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.

12.4.3 Preparation of the Jaffe washer

Set up a Jaffe washer[25] as shown in Figure 3, 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.

12.4.4 Placing of specimens into 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[13]. 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 minutes, 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 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[13].

12.5 Preparation of TEM specimens from cellulose ester analytical filters

12.5.1 Selection of area of filter for preparation

Using clean tweezers, remove the filter from the petri-dish, and place it on a cleaned microscope slide. Using a clean, curved scalpel blade, cut out a sector of the filter.

12.5.2 Preparation of solution for collapsing cellulose ester filters

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

12.5.3 Filter-collapsing procedure

Using a micropipette with a disposable tip, place 15 µl/cm^2–25 µl/cm^2 of the collapsing solution 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 degrees 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. Include a portion of an unused MEC filter as a direct-transfer blank. Place the slide either on a thermostatically-controlled slide warmer at a temperature of 65 °C to 70 °C, or in an oven 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.

12.5.4 Plasma etching of the filter surface

The conditions required in the particular plasma asher shall be established using the procedure defined 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 used. 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.

NOTE Rapid air admission can disturb particulates on the surface of the etched filter.

12.5.5 Carbon-coating

Carbon coat the microscope slide holding the collapsed filter portions as described in 12.4.2.

12.5.6 Preparation of the Jaffe washer

Set up a Jaffe washer[25] 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.

12.5.7 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.

12.6 Criteria for acceptable TEM specimen grids

Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Incomplete dispersion of the residual ash from the sample collection filter, or non-uniform deposition of particulate due to the use of incorrect filtration procedures are of major concern. Examine in the TEM all three of the TEM grids corresponding to the largest filtered aliquot at a magnification sufficiently low (300 to 1 000) so that complete grid openings can be inspected. If large areas of undispersed ash are observed on widely separated grid openings, the sample collection filter was either incompletely ashed, or the filter medium may be unsuitable for this preparation procedure. Reject all of the filters and grids. If it is suspected that the filters are unsuitable for this analytical procedure, use the procedure specified in Annex H to confirm that the filter medium is suitable for analysis by this method, and re-prepare the sample from another sector of the sample collection filter.

If the particulate deposit on the TEM grids corresponding to filtration of the largest filtered aliquot appears to be uniform, it is then necessary to select the set of TEM grids with an appropriate loading for fibre counting. Reject the grids if:

a) the carbon replica covers less than approximately 75 % of the area of the TEM specimen grids;

b) the TEM specimen has not been cleared of filter medium by the filter dissolution step. If the TEM specimen exhibits areas of undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional solvent washing shall be carried out, or new specimens shall be prepared from the analytical filter;

c) the sample is over-loaded 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 over-loaded. This filter cannot be analysed satisfactorily 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 particulate may lead to under-estimation of the structure count. Specimens prepared from a filter corresponding to filtration of a smaller aliquot shall be selected;

d) 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 caused by incorrect installation of the filters for filtration of the aqueous dispersions, or inadequate mixing during dilution of a small volume aliquot. Satisfactory analysis of this filter may not be possible unless a large number of grid openings is examined;

e) 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^2. Select specimens prepared from a filter corresponding to filtration of a smaller aliquot;

f) 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 structure count. Prepare specimens from another sector of the analytical filter, or select grids corresponding to filtration of a smaller aliquot.

If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, an additional carbon coating may be applied to the carbon coated filter, and new specimen grids prepared. The larger particles tan often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analysed, and grids prepared from an analytical filter with a lower particulate loading shall be selected.

12.7 Procedure for structure counting by TEM

12.7.1 General

The examination consists of a count of asbestos structures which are present on a specified number of grid openings. Fibrous structures are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of asbestos structures to be counted depends on the statistical precision desired. In the absence of asbestos structures, the area of the TEM specimen grids which must 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.

In order that the estimate of the structure density on the sampling filter shall not be based on the structure deposits found within the small area represented by one specimen grid, grid openings shall be examined on two of the three specimen grids prepared. The results shall then be combined for the calculation of the structure density. Structure counts shall be made at a magnification of approximately 20 000 and shall be terminated at the end of the examination of the grid opening on which the 100th primary asbestos structure is observed, except that the count shall be continued until a minimum of 4 grid openings have been examined. Otherwise, the structure count shall continue to that number of grid openings at which the specified analytical sensitivity has been achieved.

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 12.7.4 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 a larger volume aliquot or more grid openings are analysed to improve the analytical sensitivity.

12.7.2 Measurement of mean grid 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.

12.7.3 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 C.

12.7.4 Determination of criterion for termination of TEM examination

Before structure counting is begun, calculate the area of specimen to be examined in order to achieve the selected analytical sensitivity. Calculate the maximum number of grid openings to be examined from Formula (3):

$$k=\frac{\left(A_a \cdot V_d\right)}{\left(S \cdot A_g \cdot V_f \cdot F_a \cdot V_s\right)} \tag{3}$$

where

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

A_a active area of the analytical filter in mm^2;

V_d volume of water used for dispersal of residual ash in ml;

S required analytical sensitivity in structures/litre;

A_g area of TEM specimen grid opening in mm^2;

V_f volume of aqueous dispersion filtered in ml;

F_a fraction of sample collection filter ashed;

V_s volume of air sampled in litres (l).

12.7.5 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 5 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 12.7.7 for counting of 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 6. Figure 6 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 E, 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 D, 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 – 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.

12.7.6 Estimation of mass concentration of asbestos fibres and bundles

If the primary objective of the analysis is to determine an estimate of the mass concentration, it is necessary to adopt a structure counting strategy that allows large structures that contribute most to the mass concentration to be counted with greater statistical reliability. Follow the procedures described in D.6.

12.7.7 Magnification requirements

For counting of structures ≥0,5 µm in length, use a fluorescent screen magnification of approximately ×20 000. At this magnification, the image of a single 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 ×10 000 for counting of asbestos fibres and bundles longer than 5 µm, or approximately ×5 000 if only fibres and bundles longer than 5 µm and within the diameter range 0,2 µm to 3,0 µm are to be counted (PCM equivalent fibres). Use of these lower magnifications for fibres and bundles longer than 5 µm 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 µm in accordance with the procedures described in Annex F. Continue the count until completion of the grid opening on which 100 fibres and bundles have been recorded, or until 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;

b) if magnifications other than those specified in this clause are used, the minimum fibre width specified in the measurements corresponds to at least 5 pixels in the image used for fibre counting.

TEM asbestos structure count

Report Number: ______________________

Sample Number: ______________________

Sample Description: ______________________

____

____

____

____

Filename: ______________________

Preparation date: ____________ By: ____________

Analysis date: ____________ By: ____________

Computer entry date: ____________ By: ____________

Page: ________ of ____________

Air volume (l): ____________

Sample filter area (mm²): ____________

Fraction ashed: ____________

Dispersal volume (ml): ____________

Analytical filter area (mm²): ____________

Volume filtered(ml): ____________

Magnification: ____________

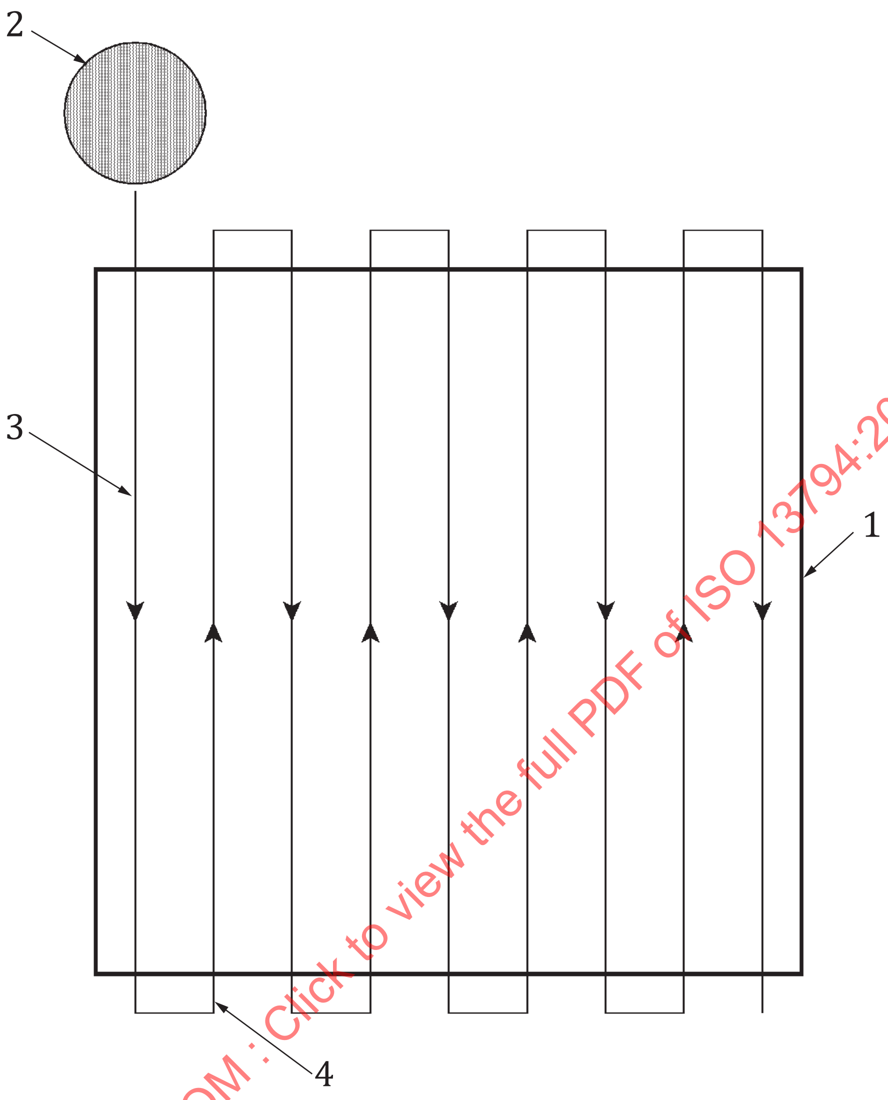
Grid opening dimension (µm): ____________

Level of analysis (chrysotile): ____________

Level of analysis (amphibole): ____________

Grid	Grid Opening	Structure		Class	Structure type	Length mm	Width mm	Fibre Identification	Comments
		Primary	Total						

Figure 5 — Example of structure-counting form



Key

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

Figure 6 — Example of scanning procedure for TEM specimen examination

12.8 Blank and quality control determinations

Before air samples are collected, analyse a minimum of two unused filters from each filter lot of 100 filters 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^2, or if the mean background fibre count for asbestos fibres longer than 5 µm is more than 0,1 fibre/mm^2, determine the origin of the contamination and correct before air samples are collected.

NOTE The background contamination originating from the sample collection filters is determined by ashing one filter, a group of 3 filters and a group of 10 filters. The background contamination originating from a single filter is calculated from any observed incremental increase between these measurements. If a constant background value is obtained for these three measurements, the contamination originates at some other point in the analytical procedure.

Include at least one open field blank and one closed field blank with the samples from each survey. To prepare an open field blank, transport an unused filter cassette to the sampling site and remove the front cap. Locate the open cassette in a similar position and orientation as the samples for the whole period of sample collection, but do not pump air through the filter. After sample collection is completed, replace the front cap and submit the cassette for analysis as the open field blank. To prepare the closed field blank, transport an unused filter cassette to the sampling site and keep it closed.

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. Process at least one funnel blank and one empty beaker blank along with each batch of samples. In addition, include at least one unused filter with every group of samples prepared on one microscope slide.

Initially, and also 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. Characterize the variability between and within microscopists and between laboratories. 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 1 or 2 structures outside of the Poisson 95 % confidence interval can be tolerated, particularly for counts of less than 20.

12.9 Calculation of results

Calculate the results using the procedures detailed in Annex G. Prior to the TEM examination of the specimens, the level of analysis was specified. Before the results are calculated, specify the compositional and morphological classifications to be included in the result. Conduct the chi-squared uniformity test using the number of primary asbestos structures found on each grid opening, prior to the application of the cluster and matrix counting criteria. Calculate the concentration result 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. The 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[36]. 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.

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 $\sqrt{N}$, 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; and

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 ±25 % when 100 structures are counted over 10 grid openings.

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[14]. 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 collected at a face velocity of 8 cm/s[15]. Using laboratory-generated and field-collected aerosols of chrysotile asbestos, it has been shown that, compared with the direct-transfer procedure, this indirect specimen preparation procedure results in comparable numbers of fibres longer than 5 µm, and less than a factor of two increase in the numbers of fibres longer than 2,5 µm. This indirect specimen preparation procedure can produce results different from those given by the direct-transfer procedure for aerosols of materials in which asbestos is blended with other components.

13.3.3 Inter- and intra-laboratory analyses

Inter- and intra-laboratory analyses are required in order to monitor systematic errors that may 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 may 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[31][33]. 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 volume of air sampled, the proportion of the sample collection filter which is ashed, the proportion of the aqueous dispersion of ash which is filtered, the area of the analytical filter, 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.

In practice, the lowest limit of detection is frequently determined by the total suspended particulate concentration, since each particle on the analytical filter has to be separated from adjacent ones by a sufficient distance that allows the particle can be identified without interference. Particulate loadings greater than about 25 µg/cm^2 on the analytical filters usually preclude preparation of TEM specimens. 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 µm, 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 to be 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), l) and m):

a) reference to this document, i.e. ISO 13794;

b) identification of the sample;

c) the date and time of sampling, and all necessary sampling data;

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 Figures E.2 and E.4);

i) a statement specifying which identification and structure categories have been used to calculate the concentration values;

j) the item:

    1) separate concentration values for chrysotile and amphibole structures equal to or longer than 0,5 µm, expressed in asbestos structures/litre;

    2) the analytical sensitivity for structures equal to or longer than 0,5 µm, expressed in asbestos structures/litre;

k) the item:

    1) separate concentration values for chrysotile and amphibole fibres and bundles longer than 5 µm, expressed in asbestos structures/litre;

  2) the analytical sensitivity for fibres and bundles longer than 5 µm, expressed in asbestos structures/litre;

l) the item:

  1) separate concentration values for chrysotile and amphibole PCM equivalent fibres, expressed in asbestos fibres/ml;

  2) the analytical sensitivity for PCM equivalent fibres, expressed in asbestos fibres/ml;

m) the mass concentration of fibres and bundles, expressed in ng/m^3.

The following items n) and o) shall be recorded, but inclusion of items n) and o) in the test report is optional:

n) a complete listing of the structure counting data; the following data should be included: grid opening number, structure number, identification category, structure type, length and width of the structure in µm, the fibre type of the structure and any comments concerning the structure;

o) compositional data or recorded EDXA spectra[5] for the principal varieties of amphibole, if present.

Examples of a suitable format for the structure counting data are shown in Figures 7 and 8.

Determination of asbestos fibres by ISO 13794

SAMPLE ANALYSIS INFORMATION

Laboratory name **Report number** **Date**

SAMPLE: Location
Location
Exterior sample 2019-03-11

Air volume:	5 750,0 l
Area of collection filter:	385,0 mm^2
Volume flowrate:	10,0 L/min
Fraction of filter ashed:	0,50
Volume of water used for dispersal of ash:	40,0 mL
Volume of dispersion filtered:	34,0 mL
Active area of analytical filter:	199,0 mm^2
Level of analysis (chrysotile):	CD or CMQ
Level of analysis (amphibole):	ADQ
Magnification used for structure counting:	20 500
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:	0,895 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:	2
Number of PCM equivalent asbestos fibres:	5

Figure 7 — Example of format for reporting of sample and preparation data

SAMPLE ANALYSIS INFORMATION (Page 2 and higher)

Laboratory name **Report number** **Date**

SAMPLE: Location
Location
Exterior sample 2019-03-11

TEM ASBESTOS STRUCTURE COUNT - RAW DATA

Grid	Grid Opening	Structures		Identification[a]	Structure type	Length µm	Width µm	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	Chrysotile	
			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	
			14	CMQ	CB	3,2	0,090	Chrysotile	
	E4-4	8	15	CD	B	1,5	0,23	Chrysotile	
		9	16	AD	F	8,7	0,15	Unidentified Amphibole	
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			No Fibres					
	E5-6	11		ADQ	CD33	9,4	5,5	Amosite	
			21	ADQ	CF	7,1	0,30	Amosite	
			22	ADQ	CF	6,2	0,10	Crocidolite	
			23	ADQ	CB	5,1	0,20	Amosite	
			24	ADQ	F	15,3	0.20	Crocidolite	
	F4-1	12	25	CMQ	MC10	3,7	2,1	Chrysotile	
		13	26	CD	CC+0	7,4	0,5	Chrysotile	

[a] Identification codes listed in Tables E.1 and E.2

Figure 8 — 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 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 plasma 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 un-collapsed 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 in 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)

Determination and standardization of operating conditions for ultrasonic bath

B.1 General

The ultrasonic bath is used for the dispersal of the residual ash from sample collection filters. Since prolonged ultrasonic treatment of asbestos dispersions can affect the size distribution of asbestos structures and thereby change the structure concentration, it is important to standardize the rate of ultrasonic energy absorption by the aqueous dispersion and the time for which the sample is exposed to the ultrasonic treatment.

Depending on the design of the ultrasonic bath, the rate of energy deposition into the contents of the sample beaker will vary with the position of the sample in the bath and the thickness of the glass. Accordingly, calibrate in the positions used, and always place the sample beakers in these positions. During operation, the temperature of the water in the ultrasonic bath will rise, and will reach an equilibrium temperature. Since the energy transfer to the sample beaker increases with the temperature of the water in the bath, operate the bath at its equilibrium temperature.

Ultrasonic energy absorbed by the sample reappears as heat, and the rate of absorption of ultrasonic energy can be measured calorimetrically. Use the measurement procedure specified in B.2.

B.2 Procedure

Ensure that the level of water in the ultrasonic bath is adjusted to that which will be used. Operate the bath until it reaches the equilibrium temperature, accelerating this process if necessary by initially using hot water in the bath. Place 40 m of water at approximately 20 °C into a 50 ml beaker, and measure its temperature. With the bath switched off, place the beaker into the position to be calibrated. After a period of 60 s to 90 s, remove the beaker and measure the temperature of the contents. Discard the contents of the beaker. Using another 40 ml of water at approximately 20 °C, measure the initial temperature of the contents, and with the ultrasonic bath operating, the final temperature when the beaker has spent in the same time in the bath as for the first measurement. Calculate the rate of energy absorption by the sample, R, expressed in watts per millilitre (W/ml), using Formula (B.1).

$$R = 4{,}185 \times \frac{(\theta_2 - \theta_1)}{t} \tag{B.1}$$

where

θ_2 is the temperature rise with ultrasonic bath operating, expressed in degrees Celsius (°C);

θ_1 is the temperature rise with the ultrasonic bath not operating, expressed in degrees Celsius (°C);

t is the time, expressed in seconds (s).

If the rate of energy absorption is higher than the specified range, use a variable transformer as the power supply for the ultrasonic bath, and adjust the operating conditions so that the rate of energy absorption is in the range 0,05 W/ml to 0,1 W/ml, as defined by this measurement procedure.

Always operate the ultrasonic bath at its equilibrium temperature, because the energy transfer to the beaker increases with temperature. If a thermometer is used for temperature measurement when calibrating the bath, do not immerse the thermometer in the beaker of water during the exposure to the bath, because the presence of the thermometer increases the absorption of energy during ultrasonic treatment. Alternatively, a low thermal capacity thermocouple may be used for temperature measurement.

Annex C

(normative)

Calibration procedures

C.1 Calibration of the TEM

C.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 10.3.13). Methods for calibration of the TEM magnification are specified in ISO 29301[8]. 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.

C.1.2 Calibration of ED camera constant

Calibrate the camera constant of the TEM when used in ED mode. 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 (C.1):

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

where

λ 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);

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

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

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

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

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

C.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[17]. 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[26] 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 mineralogical literature. 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[17].

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 by Formula (C.2) can be used to perform quantitative analyses in the TEM:

$$\frac{C_i}{C_{\mathrm{Si}}} = k_i \times \frac{A_i}{A_{\mathrm{Si}}} \tag{C.2}$$

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 2 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[30]. This 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 µm, 0,25 µm to 0,5 µm, 0,5 µm to 1,0 µm and >1,0 µm. 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 ×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 µm 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 E.

Annex D
(normative)

Structure counting criteria

D.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 D.1.

D.2 Structure definitions and treatment

D.2.1 General

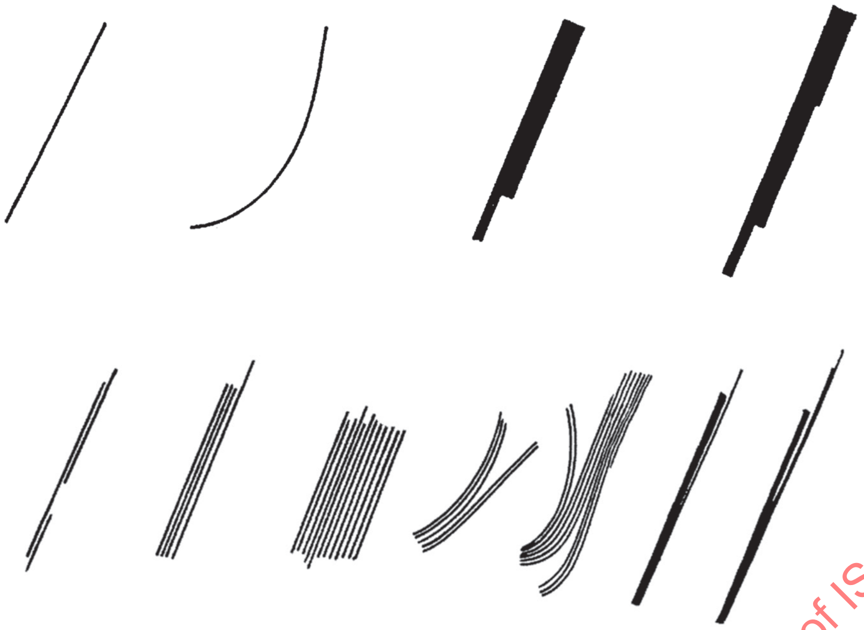
Each fibrous structure that is a separate entity shall be designated as a primary structure. Each primary structure shall be designated as a fibre, bundle, cluster or matrix. These structures are discussed in D.2.2 to D.2.9.

D.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.

D.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.



a) Fibres

b) Bundles

c) Disperse cluster (Type D)

d) Compact cluster (Type C)

e) Disperse matrix (Type D)

f) Compact matrix (Type C)

Figure D.1 — Fundamental morphological structure types

D.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 D.2.

D.2.5 Matrix

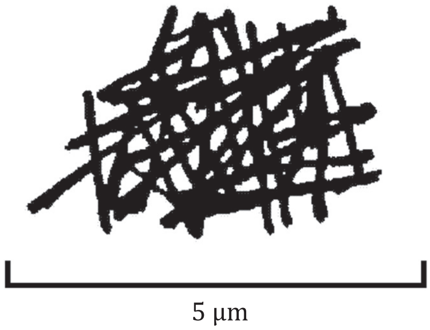
One or more fibres, or fibre bundles, may be attached to, or partially concealed by, a single particle or group of overlapping non-fibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles which are attached to fibres, and those which have by 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;

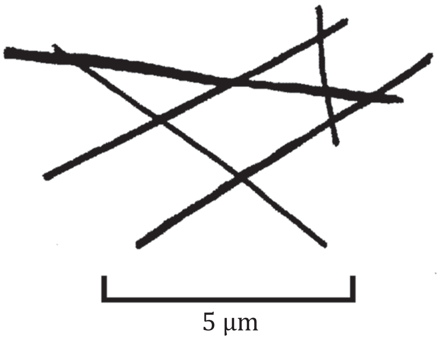
b) compact matrix (type C): a structure consisting of a particle or linked group of particles, in which fibres or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

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 D.3.



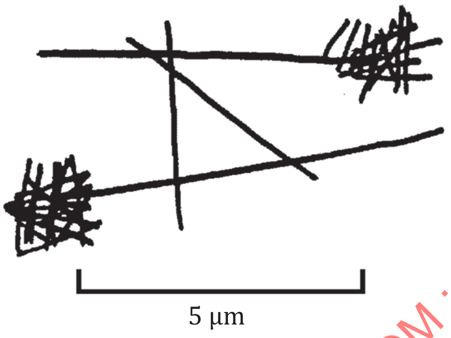
Count as 1 compact cluster (all fibres shorter than 5 µm)

Record as CC+0



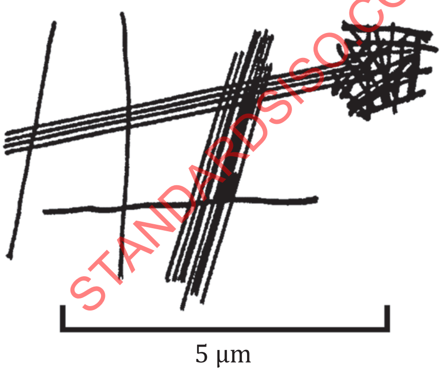
Count as a disperse cluster consisting of 5 fibres, 4 of which are longer than 5 µm

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

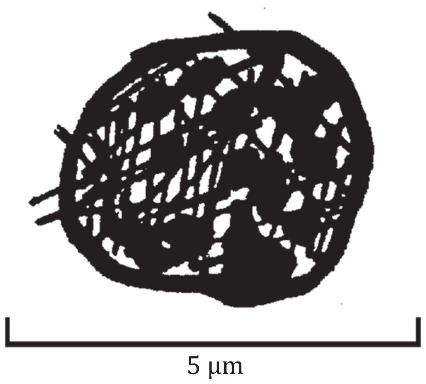
Record as CD+2, followed by 4 fibres each recorded as CF and 2 cluster residuals each recorded as CR+0



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

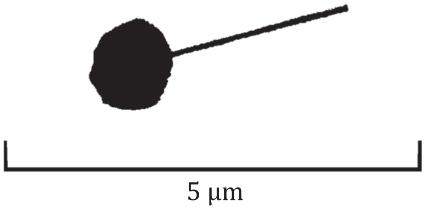
Record as CD+1, followed by 3 fibres each recorded as CF, 2 bundles each recorded as CB, and one cluster residual recorded as CR+0

Figure D.2 — Examples of recording of complex asbestos cluster



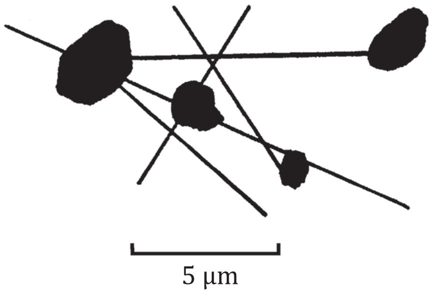
Count as one compact matrix, with all fibres shorter than 5 µm

Record as MC+0



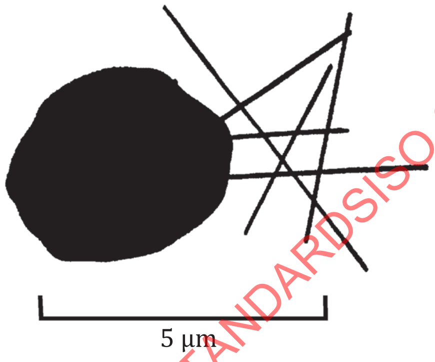
Count as 1 disperse matrix containing one fibre shorter than 5 µm

Record as MD10, followed by one fibre recorded as MF



Count as one disperse matrix containing 5 fibres, all longer than 5 µm

Record as MD55, followed by 5 fibres each recorded as MF



Count as one disperse matrix, containing 3 fibres, one of which is longer than 5 µm, and 1 matrix residual

Record as MD61, followed by 3 fibres each recorded as MF, and one matrix residual recorded as MR30

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

D.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.

D.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.

D.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.

D.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.

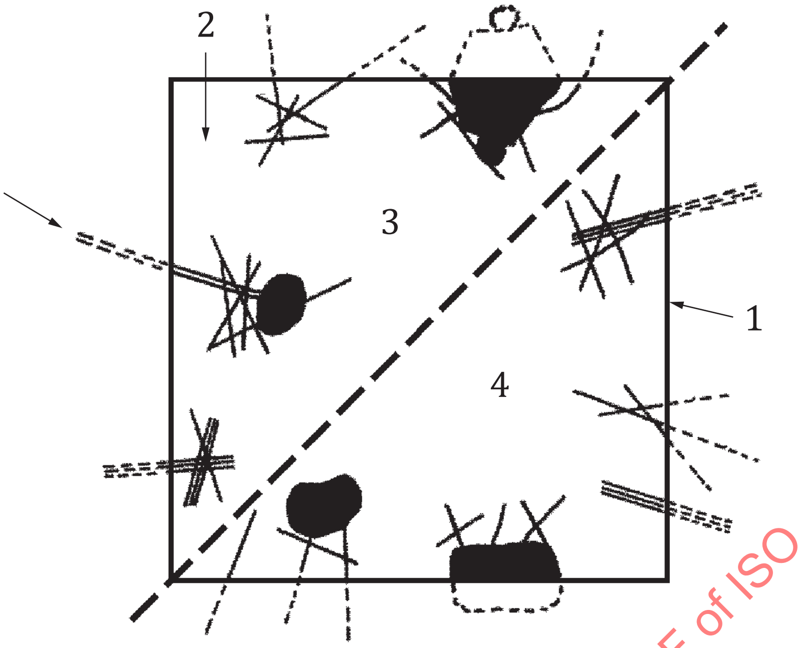
D.3 Other structure counting criteria

D.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 D.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 D.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.

D.3.2 Fibres which extend outside the field of view

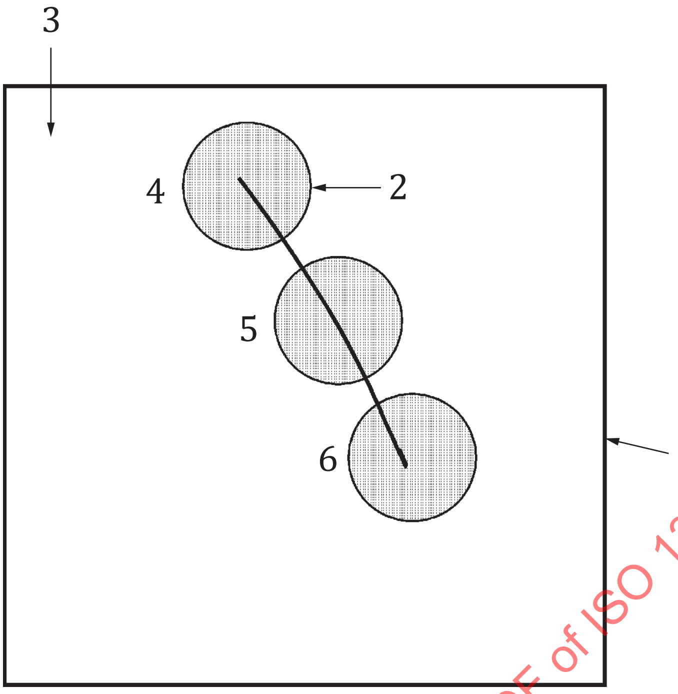
During scanning of a grid opening, count fibres which extend outside the field of view systematically, so as to avoid double-counting. In general, a rule should be established so that fibres extending outside the field of view in only two quadrants are counted. The procedure is illustrated by Figure D.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.



Key

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

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



Key

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

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

D.4 Procedure for data recording

D.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.

D.4.2 Fibres

On the structure counting form, a fibre as defined in D.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.

D.4.3 Bundles

On the structure counting form, a bundle as defined in D.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.

D.4.4 Disperse clusters (type D)

On the structure counting form, an isolated cluster of type D as defined in D.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.

D.4.5 Compact clusters (type C)

On the structure counting form, an isolated cluster of type C as defined in D.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.

D.4.6 Disperse matrices (type D)

On the structure counting form, an isolated matrix of type D as defined in D.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.

D.4.7 Compact matrices (type C)

On the structure counting form, an isolated matrix of type C as defined in D.2.5 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.

D.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.

D.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, 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.

D.6 Special considerations for determination of an estimate of the mass concentration of asbestos fibres and bundles

If the primary objective of the analysis is to determine an estimate of the mass concentration, it is necessary to adopt a structure counting strategy that allows large structures that contribute most to the mass concentration to be counted with greater statistical reliability. The number of structures that have to be counted in order to achieve a reliable estimate of the mass concentration depends primarily on the range of diameter distribution. The mass concentration measurement is most sensitive to fibres and bundles of large diameters, which are generally statistically infrequent relative to the smaller fibres and bundles. If the diameter distribution is narrow, such as that found in a dispersion of chrysotile fibrils, the mass concentration can be measured with approximately the same precision as that of the numerical concentration. If the diameter distribution is broad, mass concentration estimates derived from TEM examinations to determine numerical concentrations are statistically unreliable. The strategy specified below is designed to give greater statistical significance to the large structures, which contribute most to the mass concentration.

Initially, establish the largest width of asbestos fibre or bundle that can be detected on the grid by a cursory survey, at a reduced magnification, of approximately 50 grid openings. Calculate the volume of this structure. Adjust the magnification of the TEM to a value such that a width of 1 mm on the fluorescent screen corresponds to approximately 10 % of the width of the previously selected large structure. Carry out a routine TEM examination at this magnification, terminating the examination at the end of the grid opening on which the integrated volume of all structures recorded is at least 10 times the volume of the originally selected structure. For asbestos structures which intercept the grid bars, measure only the unobscured parts of the fibres and bundles within the grid opening, for the purposes of calculation of the mass concentration of asbestos. Disregard the procedure indicated in D.3.1 and Figure D.4.

Annex E
(normative)

Fibre identification procedure

E.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 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.

E.2 ED and EDXA techniques

E.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.

E.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[19], P.B. Hirsch, et al.[21], and H.R. Wenk[35], 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 E.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:

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

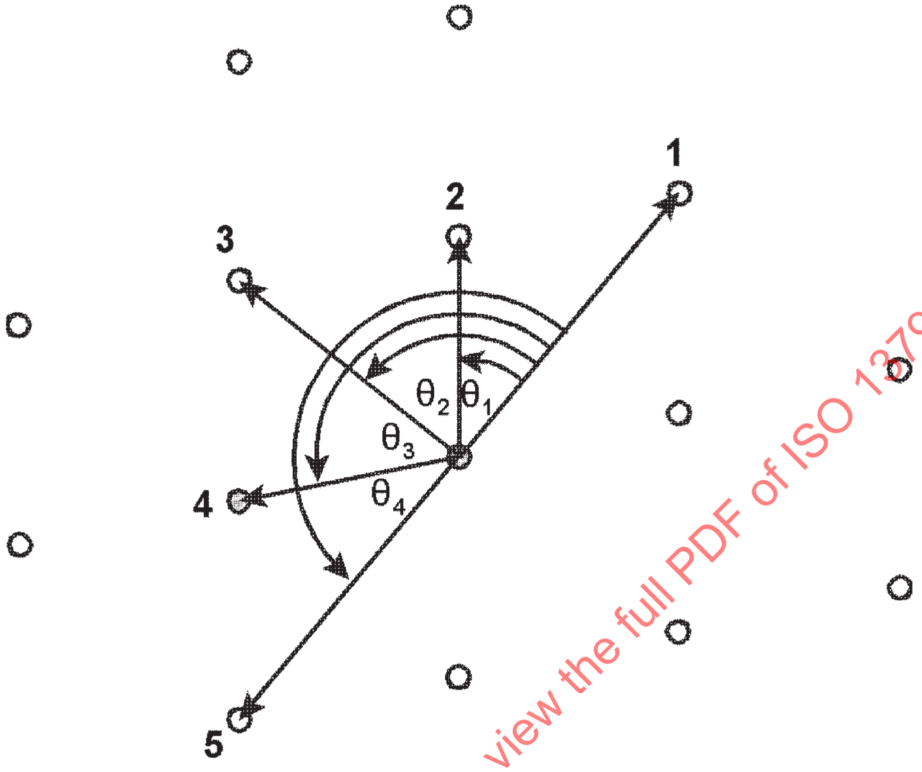


Figure E.1 — Example of measurement of zone-axis SAED patterns

E.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 Kα 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.

E.3 Interpretation of fibre analysis data

E.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.

E.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[29] are recommended for interpretation of zone-axis ED patterns. The published literature[9][19] contains composition and crystallographic data for all of 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 E.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.

E.4 Fibre classification categories

E.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 E.1 and E.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 E.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 E.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

E.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 E.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 E.2. Where the morphology is more definitive, it may be possible to classify the fibre as having chrysotile morphology (CM).

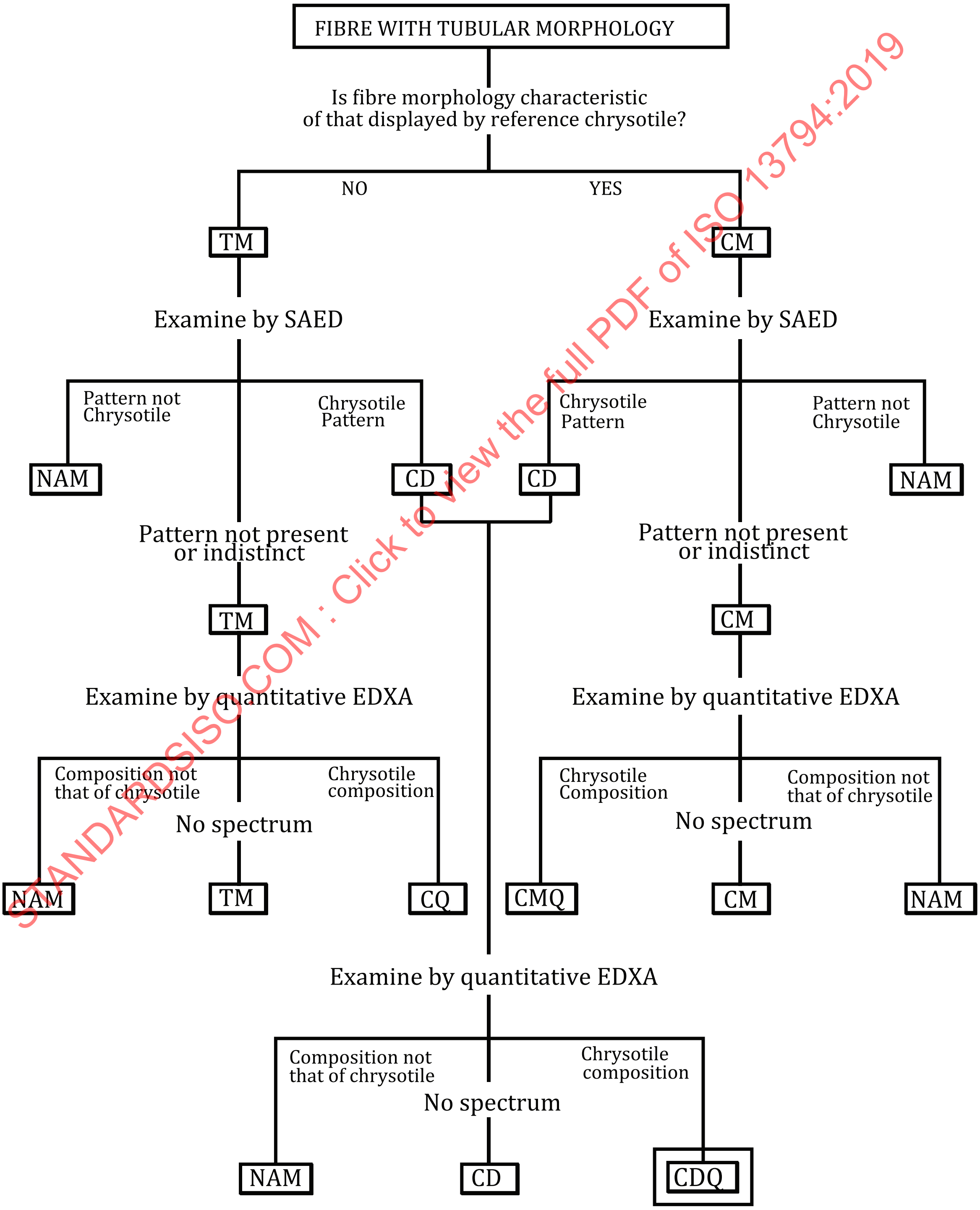


Figure E.2 — Classification chart for fibre with tubular morphology

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 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 E.3. Since chrysotile has cylindrical symmetry[37], the ED pattern remains unchanged during 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;
- there should be “streaking” of the (110) and (130) reflections.

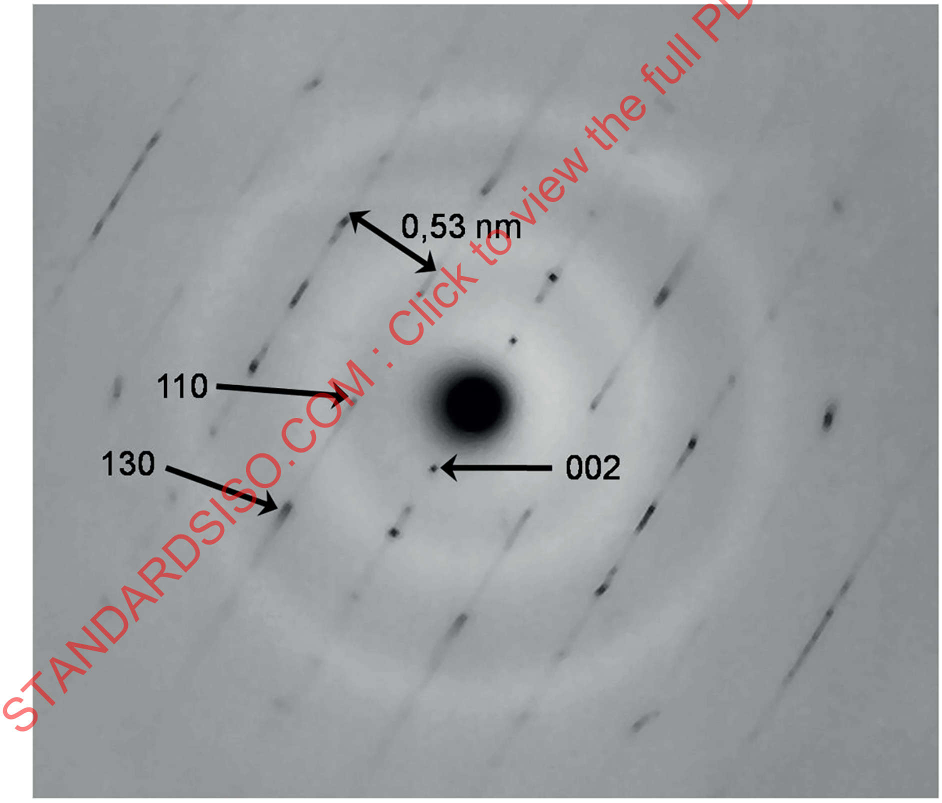


Figure E.3 — Chrysotile SAED pattern

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[16] or lizardite[36]. 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 shall 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.

E.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 E.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.

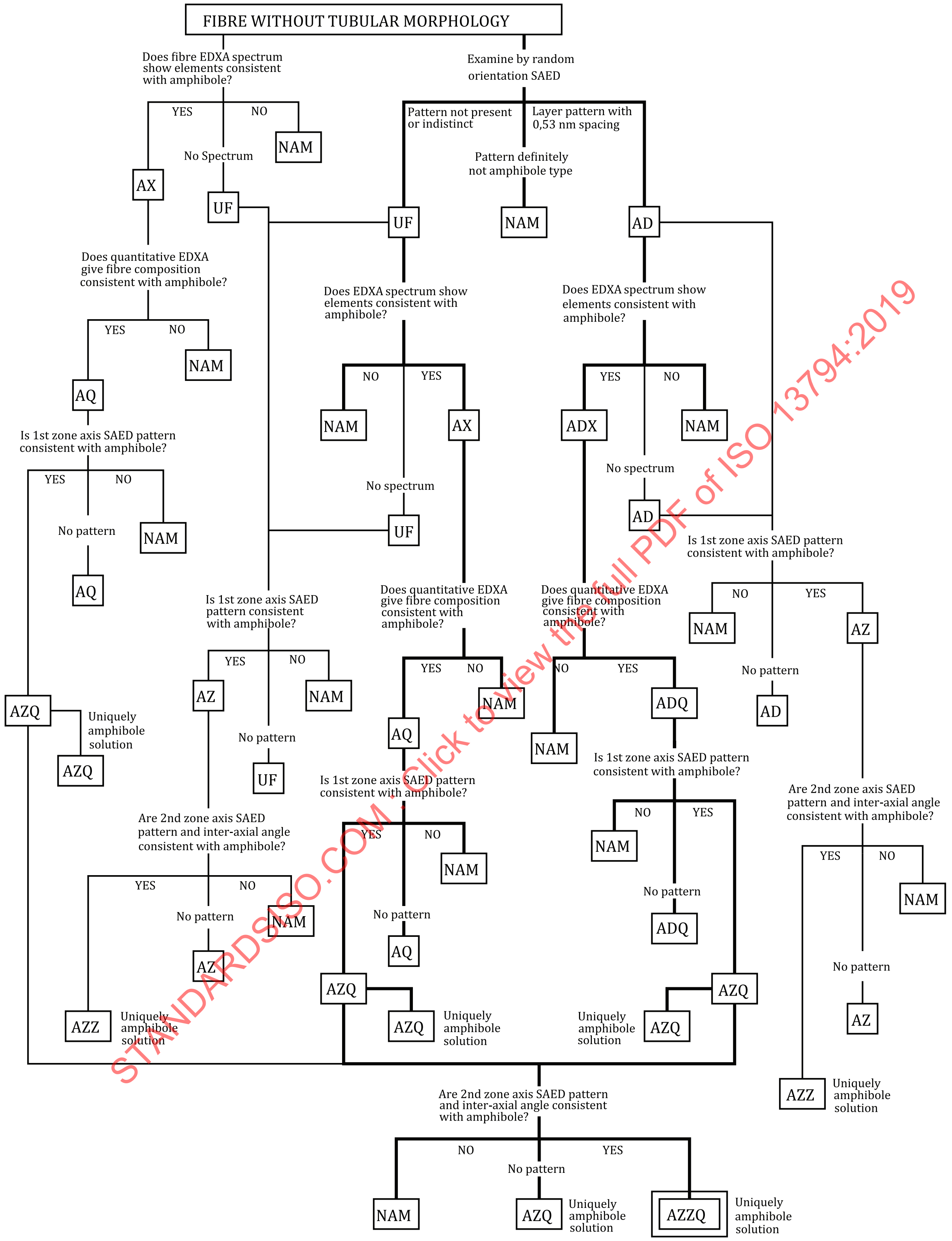


Figure E.4 — Classification chart for fibre without tubular morphology

E.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 E.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.

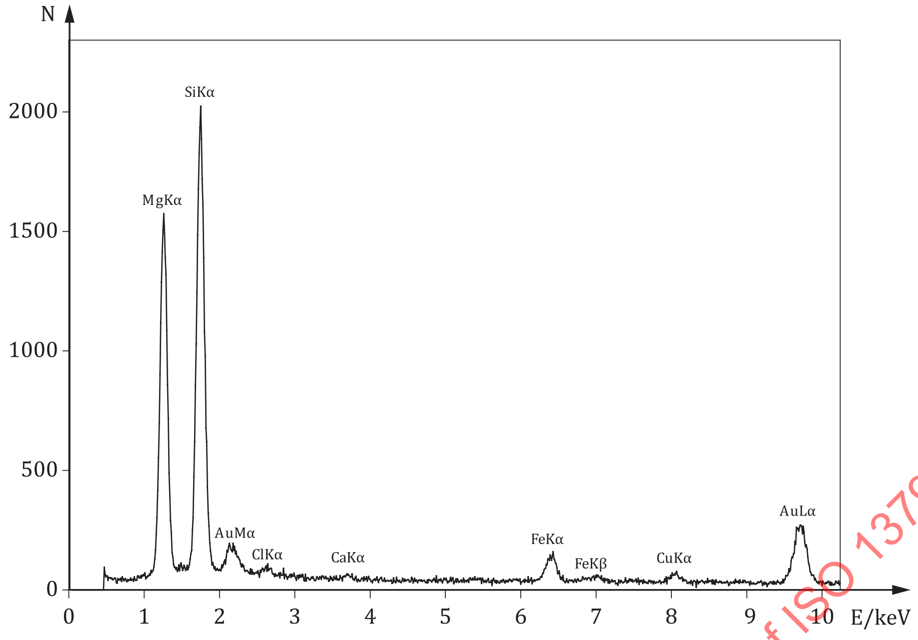
The reference EDXA spectra that follow are shown as examples only. They were acquired from TEM specimens prepared on gold grids, using an X-ray detector with a beryllium window. X-ray detectors with ultra-thin windows produce EDXA spectra with higher efficiency for elements of low atomic number. It is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

Reference EDXA spectra for NIST SRM 1866 chrysotile, amosite and crocidolite are shown in Figures E.5 to E.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 E8.

Figure E.9 shows an EDXA spectrum from NIST SRM 1867 anthophyllite, and Figure E.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 E.9 and E.10. In some sources of anthophyllite, iron is not detectable by EDXA.

Figures E.11 and E.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 E.13, is an example of tremolite with a lower iron concentration. The EDXA spectrum from HSE reference actinolite, shown in Figure E.14, is an example of actinolite with a high iron concentration.

Figure E.15 shows an EDXA spectrum obtained from richterite-winchite. This mineral is associated with some sources of vermiculite. Figure E.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.



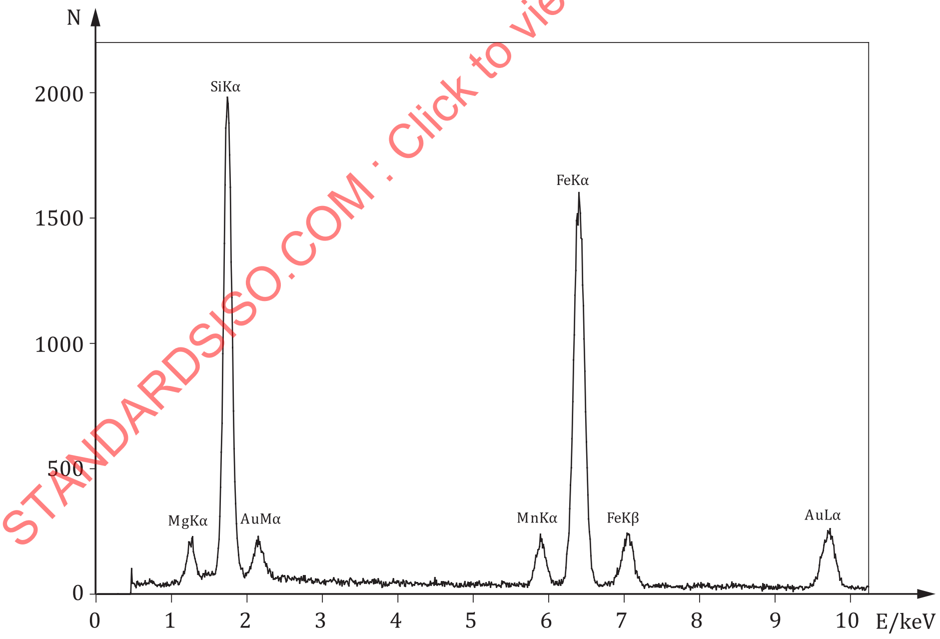
Key

N counts

E X-ray energy

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

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



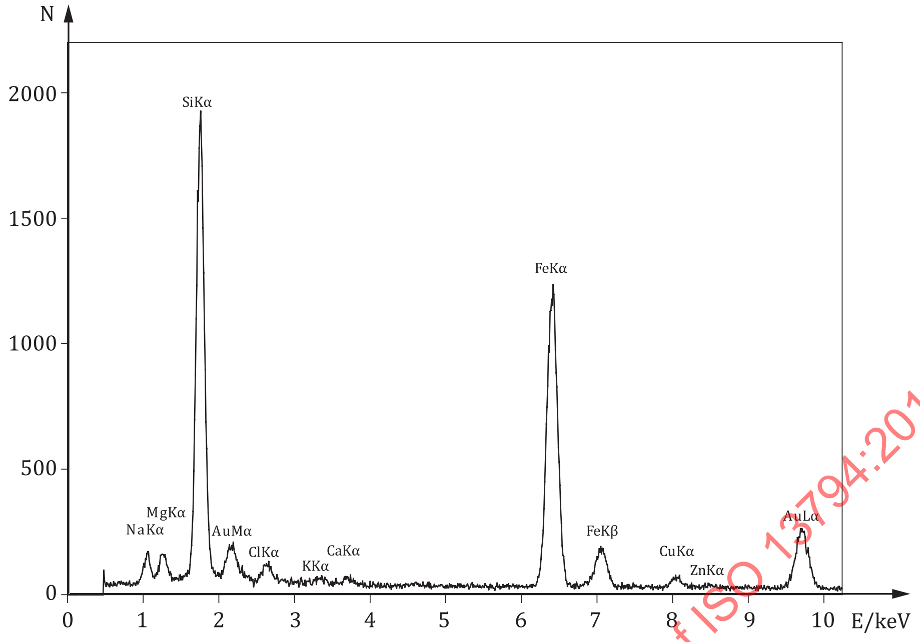
Key

N counts

E X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

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

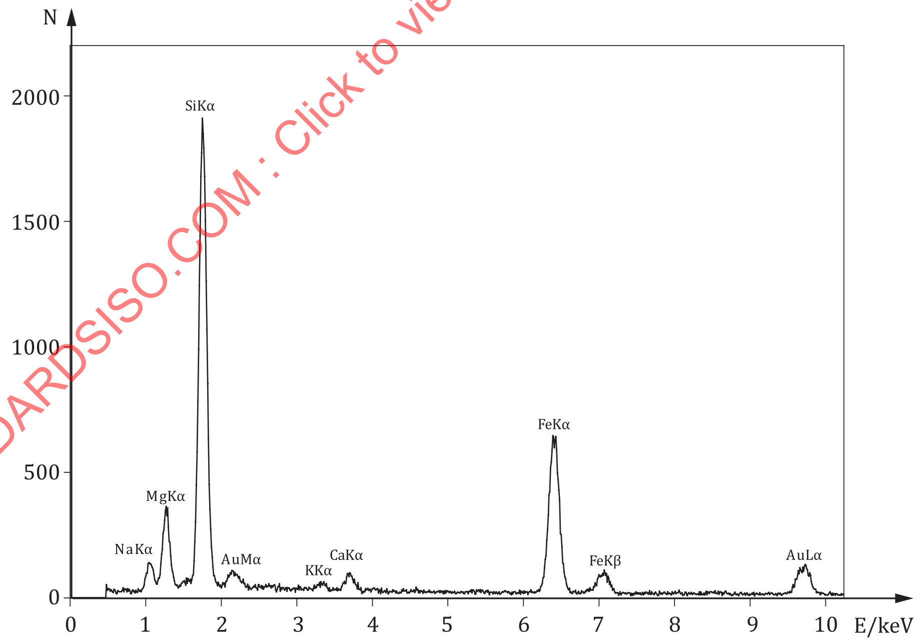


Key

N	counts	*E*	X-ray energy

NOTE 1 The gold and small copper peaks originate from the gold specimen grid.

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

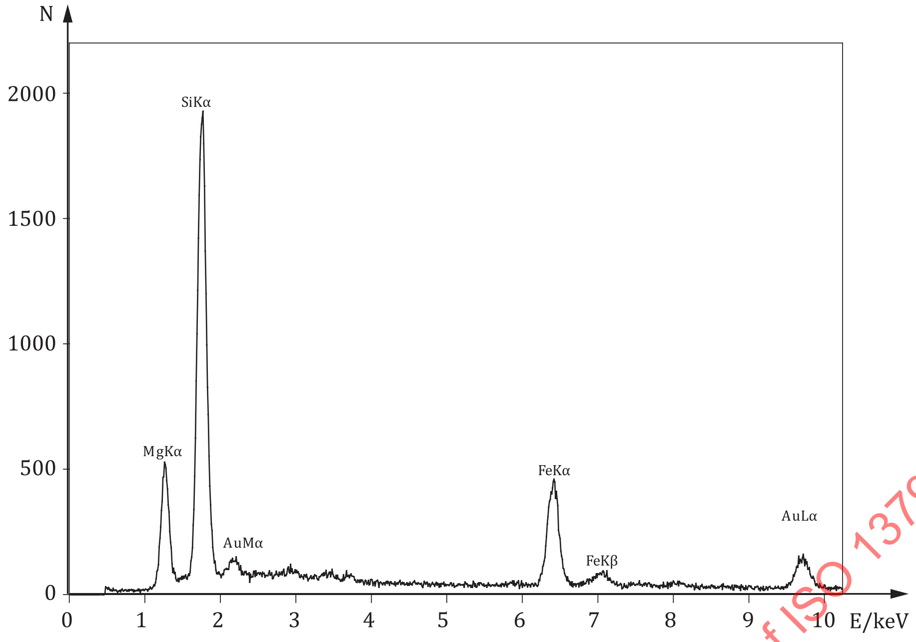


Key

N	counts	*E*	X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

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



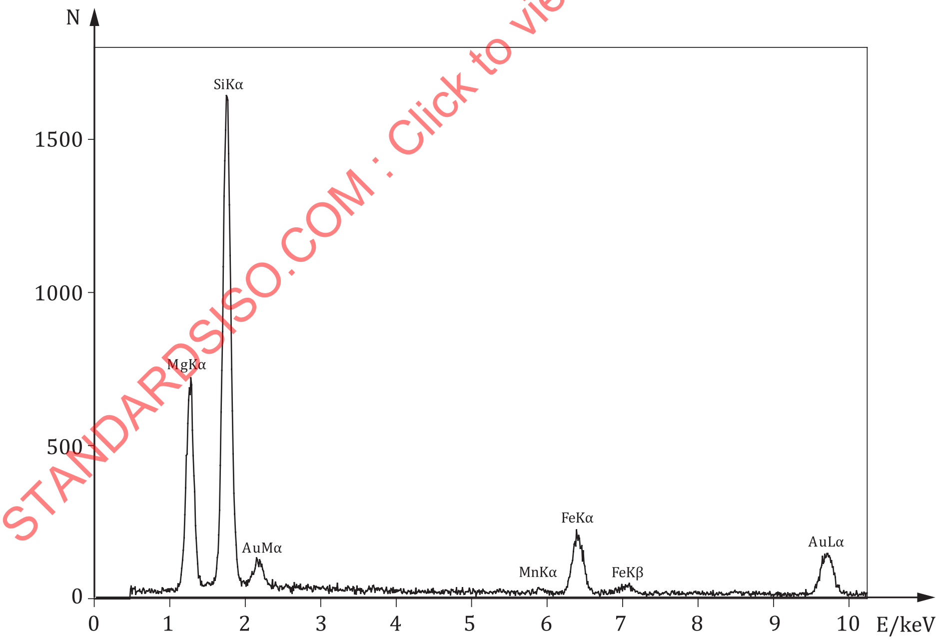
Key

N counts

E X-ray energy

NOTE The gold peaks originate from the gold specimen grid.

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