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**Measuring method for airborne fibrous
particles — Part 3: Indirect-transfer
transmission electron microscopy
method**

ICS 13.040.20

Descriptors : air, quality, exhaust gases, asbestos, determination of content, particle size measurement, coarse-grain material, permeability measurement, electron microscopes

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Foreword

This translation has been made based on the original Japanese Industrial Standard established by the Minister of International Trade and Industry through deliberations at the Japanese Industrial Standards Committee in accordance with the Industrial Standardization Law.

JIS K 3850 series consist of the following four parts with the title of *Measuring method for airborne fibrous particles*.

Part 1: *Optical microscopy method and scanning electron microscopy method*

Part 2: *Direct-transfer transmission electron microscopy method*

Part 3: *Indirect-transfer transmission electron microscopy method*

Part 4: *Stationary source emissions — Determination of asbestos plant emissions — Method by fibre count measurement*

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Measuring method for airborne fibrous particles — Part 3: Indirect-transfer transmission electron microscopy method

Introduction This Japanese Industrial Standard has been prepared based on **ISO/DIS 13794** *Ambient air—Determination of asbestos fibres—Indirect-transfer transmission electron microscopy method* published in 1996 without modifying the technical contents.

The portions underlined with dots are the matters not stated in the original International Standard.

This Standard is applicable to the measurement of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for detailed evaluation of any atmosphere in which asbestos fibres are likely to be present. 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 Standard is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. 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 TEM preparations. The method defined in this Standard incorporates specimen preparation procedures that result in selective concentration of asbestos fibres, and removal of organic and water-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 Standard is necessarily complex, because the instrumental techniques used are complex, and also because a very detailed and logical procedure must be specified to reduce the subjective aspects of the measurement. The method of data recording specified in this Standard is designed to allow re-evaluation of the fibre counting data as new medical evidence becomes available.

This Standard 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 may not be done a priori. A site-specific intercomparison study must be done which takes into account the fibre size and type of asbestos, and also the nature of the source of the airborne asbestos.

1 Scope

1.1 Substance determined This Standard specifies a reference method using transmission electron microscopy (TEM) for determination of the concentration of asbestos structures in ambient atmospheres. 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 fibre present. The method cannot discriminate between individual fibres of the asbestos and non-asbestos analogues of the same amphibole mineral.

1.2 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. The method is suitable for determination of asbestos in both exterior and building atmospheres.

1.3 Range The upper limit for the range of concentration that can be measured on the analytical filter is 7 000 structures/mm². The lower limit of the range that can be measured on the analytical filter corresponds to detection of 2.99 structures in the area of specimen examined. 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 for measurements in areas with high suspended-particulate concentrations (exceeding 10 µg/m³), or where detection and identification of asbestos fibres are likely to be prevented or hindered by other types of particulate in direct-transfer TEM preparations. In theory, there is no lower limit to the dimensions of asbestos fibres which can be detected. In practice, microscopists vary in their ability to detect very small asbestos fibres. Therefore, a minimum length of 0.5 µm has been defined as the shortest fibre to be incorporated in the reported results.

1.4 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 TEM specimen examined 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 $10 \mu\text{g}/\text{m}^3$, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 litres of air, an analytical sensitivity of 0.5 structure/litre can be obtained, equivalent to a limit of detection of 1.8 structures/litre, if an area of 0.195 mm^2 of the TEM specimens is examined. Lower limits of detection can be achieved by increasing the area of the TEM specimen 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 $5 \mu\text{m}$, 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.

2 Normative references The following standards contain provisions which, through reference in this Standard, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 3850-2 *Measuring method for airborne fibrous particles—Part 2: Direct-transfer transmission electron microscopy method*

NOTE: ISO 10312:1995 *Ambient air—Determination of asbestos fibres—Direct-transfer transmission electron microscopy method* is identical with the said standard.

ISO 4225 *Air quality—General aspects—Vocabulary*

ISO 4226 *Air quality—General aspects—Units of measurement*

ISO 5725 *Precision of test methods—Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*

ISO 7708 *Air quality—Particle size fraction definitions for health-related sampling*

ISO STANDARDS HANDBOOK 2 *Units of measurement*

ISO STANDARDS HANDBOOK 3 *Statistical Methods*

3 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 $0.4 \mu\text{m}$ or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size $0.8 \mu\text{m}$ by means of a battery-powered or mains-powered pump. A portion of the filter is ashed in an oxygen plasma, 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 PC membrane filters of maximum pore size $0.2 \mu\text{m}$ or cellulose ester membrane filters of maximum pore size $0.22 \mu\text{m}$.

TEM specimens are prepared from PC analytical filters by applying a thin film of carbon 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 of carbon which bridges the openings in the TEM specimen grid, and which supports each particle from the original 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 to ensure that all particles are exposed. 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) is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy-dispersive X-ray analysis (EDXA). 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 ED pattern, and the qualitative and quantitative EDXAs. 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.

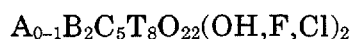
A coding system is used to record the type of fibrous structure, and also to provide the optimum morphological description of each structure. The two codes remove from the microscopist the requirement to interpret the fibre counting data, and allow this evaluation to be made later without the requirement 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, expressed 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.

4 Terms and definitions For the purposes of this Standard the terms and definitions given in **ISO 4225**, and the following terms and definitions apply.

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

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



where A is K, Na

B is Fe²⁺, Mn, Mg, Ca, Na

C is Al, Cr, Ti, Fe³⁺, Mg, Fe²⁺

T is Si, Al, Cr, Fe³⁺, Ti

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°.

4.3 amphibole asbestos amphibole in an asbestiform habit

4.4 analytical filter filter through which an aqueous dispersion of ash from the sample collection filter is passed, and from which TEM specimen grids are prepared

Informative reference: **4.4A analytical sensitivity** calculated airborne asbestos structure concentration, equivalent to counting of one asbestos structure in the analysis. It is expressed in structures/litre. The method given in this Standard does not specify an analytical sensitivity.

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

4.6 asbestos term applied to a 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. 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).

4.7 asbestos structure term applied to an individual asbestos fibre, or any connected or overlapping grouping of asbestos fibres or bundles, with or without other particles

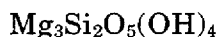
4.8 ashed filter blank fibre count made on TEM specimens prepared by the indirect procedure from a blank membrane filter of the type used for collection of air samples

4.9 aspect ratio ratio of length to width of a particle

4.10 blank structure count made on TEM specimens prepared from an unused filter in order to determine the background measurement

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

4.12 chrysotile fibrous mineral of the serpentine group which has the nominal composition



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.

4.13 cleavage breaking of a mineral along one of its crystallographic directions

4.14 cleavage fragment fragment of a crystal that is bounded by cleavage faces

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

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

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

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

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

4.20 empty beaker blank fibre count made on TEM specimens prepared by the indirect procedure, using an empty beaker as the initial sample

4.21 energy-dispersive X-ray analysis EDXA Measurement of the energies and intensities of X-rays by use of a solid-state detector and multichannel analyzer system

4.22 eucentric condition in which 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

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

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

4.25 fibre elongated particle which has parallel or stepped sides. For the purposes of this Standard, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0.5 µm.

4.26 fibre bundle structure composed of parallel, smaller-diameter fibres attached along their lengths. A fibre bundle may exhibit diverging fibres at one or both ends.

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

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

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

4.30 limit of detection calculated airborne asbestos structure concentration, equivalent to counting of 2.99 asbestos structures in the analysis. It is expressed in structures/litre.

4.31 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 nonfibrous particles

4.32 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

4.33 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

NOTE: For the purposes of this Standard, PCM is the abbreviated term for phase-contrast optical microscopy.

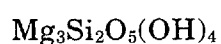
4.34 PCM-equivalent structure fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0.2 µm and 3.0 µm

4.35 primary structure fibrous structure that is a separate entity in the TEM image

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

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

4.38 serpentine group of common rock-forming minerals having the nominal formula



4.39 structure single fibre, fibre bundle, cluster or matrix

4.40 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

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

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

5 Symbol of units and abbreviated terms (refer to ISO 4226 and ISO No. 2)

5.1 Units

eV	electron volt
kV	kilovolt
L/min	litres per minute
µg	microgram (10^{-6} gram)
µm	micrometre (10^{-6} metre)
nm	nanometre (10^{-9} metre)
W	watt

5.2 Abbreviated terms

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

6 Apparatus

6.1 Air sampling

6.1.1 Filter cassette, for sample collection. Field monitors, comprising 25 mm to 50 mm diameter three-piece cassettes with cowls which project less than 2 cm in front of the filter surface, shall be used for sample collection. The cassette shall be loaded with either a capillary pore PC filter of maximum pore size 0.4 μm or a cellulose ester [mixed esters of cellulose (MEC) or cellulose nitrate] filter of maximum pore size 0.8 μm . 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.

NOTE: Representative filters from the filter lot shall be analyzed as described in 10.8 for the presence of asbestos structures, and also tested for suitability as described in annex H, before any are used for air sample collection.

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 in JIS K 3850-2. The smaller maximum pore size is specified in JIS K 3850-2 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.

Representative filters from the filter lot shall be analyzed as described in 10.8 for the presence of asbestos structures, and also tested for suitability as described in annex H, before any are used for air sample collection.

6.1.2 Sampling pump Capable of a flowrate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4.0 cm/s and 70 cm/s. The sampling pump used shall provide a nonfluctuating air flow through the filter, and shall maintain the initial volume flowrate to within $\pm 10\%$ throughout the sampling period. A constant-flow or critical-orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flowrate 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 may vary with face velocity.

6.1.3 Stand, to hold the filter cassette at the desired height for sampling, isolated from the vibrations of the pump.

6.1.4 Variable area flowmeter calibrated, with a range suitable for determination of the selected flowrate as required for calibration of the air sampling system.

6.2 Specimen preparation laboratory Asbestos, particularly chrysotile, is present in varying quantities in many 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 10.8. A minimum facility considered suitable for preparation of TEM specimens is a positive pressure, laminar flow hood. 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. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE: It is recommended that activities involving manipulation of bulk asbestos samples not be performed in the same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

6.3 Equipment for analysis

6.3.1 Transmission electron microscope, operating at an accelerating potential of 80 kV to 120 kV, 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 direct screen magnification of about 100 000× is necessary for inspection of fibre morphology; this magnification may be obtained by supplementary optical enlargement of the screen image with a binocular if it cannot be obtained directly. The viewing screen shall be calibrated (such as shown in Figure 1) with concentric circles and a millimeter scale such that the lengths and widths of fibre images down to 1 mm width can be measured in increments of 1 mm.

For Bragg angles less than 0.01 rad the TEM shall be capable of performing ED from an area of 0.6 μm² 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 an individual instrument may normally be calculated using the following relationship:

$$A = 0.785 \, 4 \times (D/M + 2 \, 000 \times C_s \times \theta^3)^2$$

Where	<i>A</i>	is the effective SAED area, expressed in square micrometres (μm ²);
	<i>D</i>	is the diameter of the SAED aperture, expressed in micrometres;
	<i>M</i>	is the magnification of the objective lens;
	<i>C_s</i>	is the spherical aberration coefficient of the objective lens, expressed in millimetres;
	<i>θ</i>	is the maximum required Bragg angle, expressed in radians.

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

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

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

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

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

NOTE: Use of an anticontamination trap around the specimen is recommended if the required instrumental performance is to be obtained.

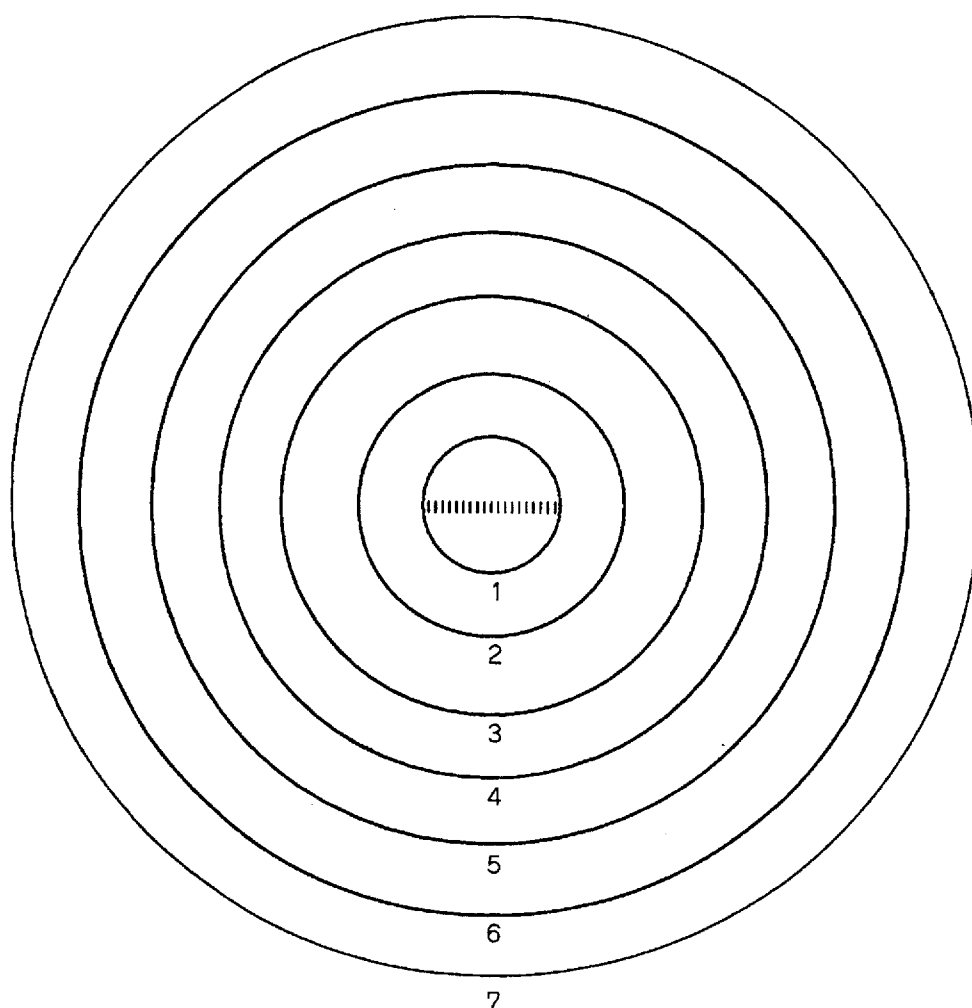


Figure 1 – Example of calibration markings on TEM viewing screen

6.3.2 Energy-dispersive X-ray analyzer, capable of achieving a resolution better than 180 eV (FWHM) on the $Mn\alpha$ peak. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of geometrical factors, the required performance of the combination of the TEM and X-ray analyzer is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known electron-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 analyzer shall yield, under routine analytical conditions, a background-subtracted $NaK\alpha$ integrated peak count rate of more than 1 count per second (cps) from a fibre of UICC crocidolite 50 nm in diameter or smaller when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1.0.

The EDXA unit shall provide the means for subtraction of the background, identification of element peaks, and calculation of background-subtracted peak areas.

6.3.3 Computer Many repetitive numerical calculations are necessary, and these may be performed conveniently by relatively simple computer programs. For analyses of zone-axis ED pattern measurements, a computer with adequate memory is required to accommodate the more complex programs involved.

6.3.4 Plasma asher, with a radio frequency power rating of 50 W or higher, for ashing of sample collection filters and for preparation of TEM specimens from 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 the particulate.

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

6.3.5 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.

NOTE: A mechanism which allows the rotating slide also 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.

6.3.6 Sputter coater (optional), with a gold target, for deposition of gold onto TEM specimens as an internal calibration of ED patterns. Other calibration materials are acceptable. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

6.3.7 Beakers, of borosilicate glass, 50 ml capacity, as containers for plasma ashing of sample collection filters.

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

6.3.9 Glass filtration apparatus, 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.

6.3.10 Solvent washer (Jaffe washer), 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.

In general, either chloroform or 1-methyl-2-pyrrolidone is used for dissolving PC filters and dimethylformamide or acetone is used for dissolving cellulose ester 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, therefore much smaller volumes of these solvents may be used. It is recommended that all washers be used in a fume hood, and when specimens are not being inserted or removed, during the solvent dissolution the Petri dish lid shall be in place. The washer shall be cleaned before it is used for each batch of specimens.

6.3.11 Condensation washer, for more rapid dissolution of the filter polymer, or if difficulties are experienced in dissolving the filter polymer. The washer shall consist of a flask, condenser and cold-finger assembly, with a heating mantle and means for controlling the temperature. A suitable assembly is shown in Figure 4, for use with either acetone or chloroform as the solvent, depending on the type of filter.

6.3.12 Slide warmer or oven, for heating slides during the preparation of TEM specimens from cellulose ester filters. It shall be capable of maintaining a temperature of 65°C to 70°C.

6.3.13 Ultrasonic bath, for aqueous dispersal of ash from sample collection filters, and also for cleaning of apparatus used for TEM specimen preparation. The power of the ultrasonic bath shall be sufficient to allow absorption of energy into a 40 ml volume of water in a 50 ml glass beaker at a rate between 0.05 W/ml to 0.1 W/ml, at a frequency of approximately 50 kHz.

6.3.14 Carbon-grating replica, with about 2 000 parallel lines per millimetre, to calibrate the magnification of the TEM.

6.3.15 Calibration grids for EDXA, prepared from dispersions of calibration minerals, for calibration of the EDXA system. Examples of suitable calibration minerals are riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. Prepare the mineral used for calibration of the EDXA system for sodium using a gold TEM grid.

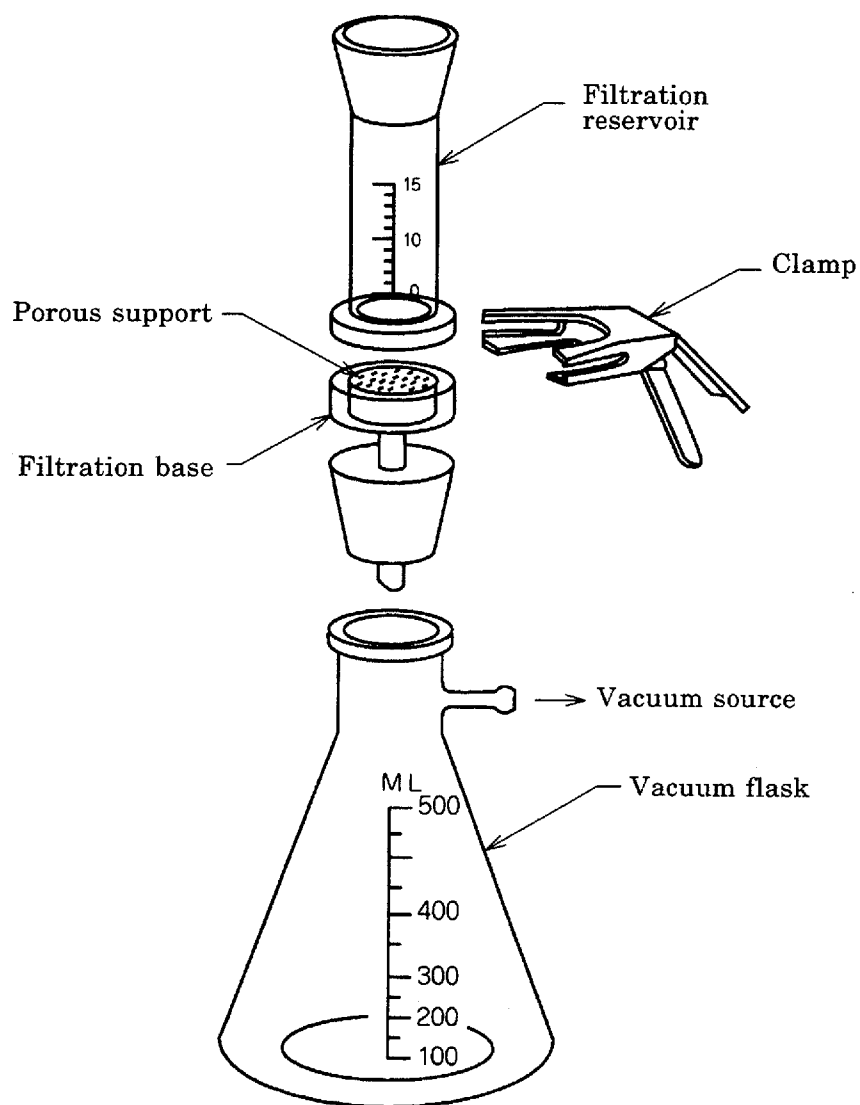
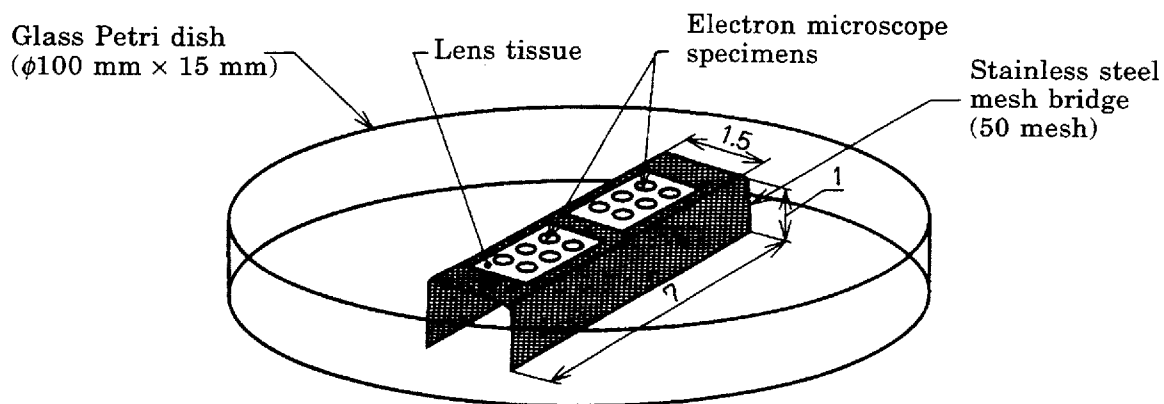


Figure 2 - Example of filtration apparatus



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

Figure 3 - Example of design of solvent washer (Jaffe washer)

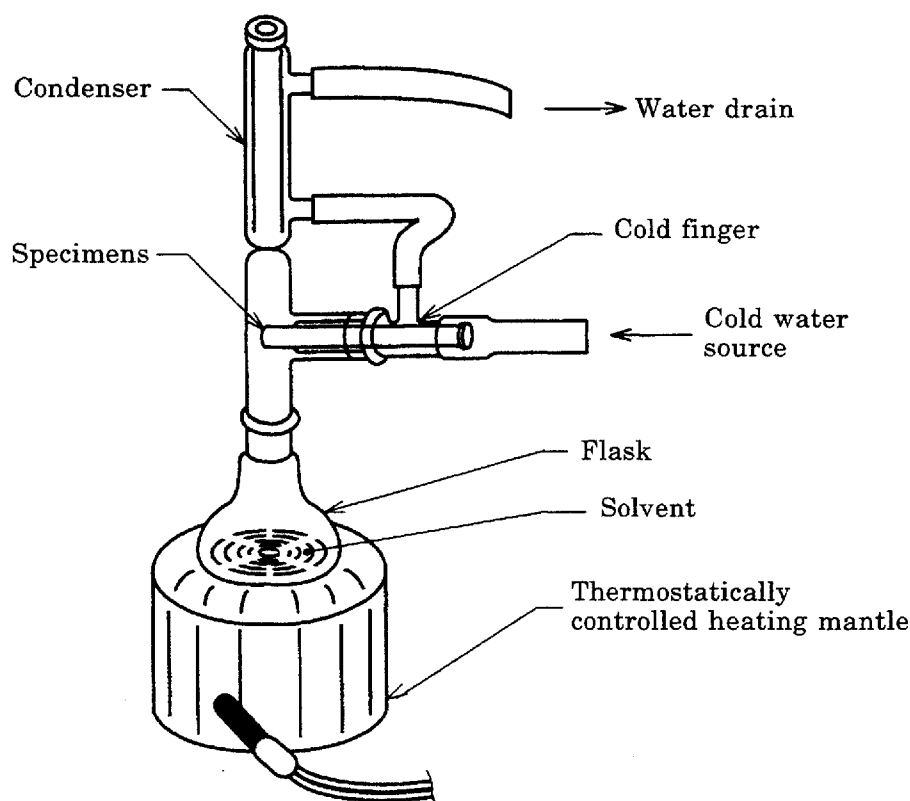


Figure 4 – Design of condensation washer

6.3.16 Carbon rod sharpener The use of necked carbon rods, or equivalent, allows the carbon to be evaporated on to the filters with a minimum of heating.

6.3.17 Disposable-tip micropipettes, capable of transferring a volume of approximately 50 μl for the pH adjustment, and a volume of approximately 30 μl for the preparation of TEM specimen grids from cellulose ester filters.

6.3.18 Thermometer, with a range from 0°C to 100°C, for calibration of the ultrasonic bath.

6.3.19 Stopwatch, for calibration of the ultrasonic bath.

6.4 Consumable supplies

6.4.1 Cellulose ester filters (either MEC or cellulose nitrate) filters of maximum pore size 0.22 μm and diameter appropriate for the liquid filtration apparatus, for filtration of the aqueous suspension of residual ash.

6.4.2 Polycarbonate filters, of maximum pore size 0.2 μm and diameter appropriate for the liquid filtration apparatus, for filtration of the aqueous suspension of residual ash.

6.4.3 Cellulose ester filters (either MEC or cellulose nitrate) of 5 μm pore size and diameter appropriate for the liquid filtration apparatus, for use with the liquid filtration apparatus.

6.4.4 Petri dishes, for storage of analytical filters prepared by filtration of the suspension of residual ash.

6.4.5 Copper electron-microscope grids Two hundred mesh TEM grids are recommended. Grids which have grid openings of uniform size such that they meet the requirement of **9.7.2** 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.

6.4.6 Gold electron-microscope grids Two-hundred-mesh gold TEM grids 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 of **9.7.2** 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.

6.4.7 Aluminium foil, for covering beakers during the plasma ashing and ultrasonic treatment steps. Aluminium foil of 0.015 mm to 0.020 mm thickness is suitable.

6.4.8 Carbon rod electrodes, spectrochemically pure, for use in the vacuum evaporator during carbon coating of filters.

6.4.9 Routine electron microscopy tools and supplies, including fine-point tweezers, scalpel holders and blades, microscope slides, double-sided adhesive tape, lens tissue, gold wire, tungsten filaments and other routine supplies are required.

6.4.10 Reference asbestos samples, for preparation of reference TEM specimens of the primary asbestos minerals. The UICC set of minerals is suitable for this purpose.

7 Reagents

Warning: Use the reagents in accordance with the appropriate health and safety regulations.

7.1 Fibre-free water, freshly distilled, or other source of fibre-free, pyrogen-free water shall be used.

7.2 Chloroform, analytical grade, distilled in glass [preserved with 1% (volume fraction) ethanol].

7.3 1-Methyl-2-pyrrolidone, analytical grade.

7.4 Dimethylformamide, analytical grade.

7.5 Glacial acetic acid, analytical grade.

7.6 Acetone, analytical grade.

8 Air sample collection

8.1 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 number of grid openings, k , required to be examined to achieve a particular analytical sensitivity is calculated using the formula:

$$k = \frac{(A_s \times V_d)}{(S \times A_g \times V_f \times F_a \times V_s)}$$

where	A_s	is the active area, expressed in square millimetres, of analytical filter;
	V_d	is the volume, expressed in millilitres, of water used for dispersal of residual ash;
	S	is the analytical sensitivity, expressed in structures/litre;
	A_g	is the mean area of grid openings, expressed in square millimeters;
	V_f	is the volume, expressed in millilitres, of aqueous dispersion filtered;
	F_a	is the fraction of sample collection filter ashed;
	V_s	is the volume, expressed in litres, of air sampled.

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

Air samples shall be collected using cassettes as described in 6.1.1. During 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. Measure the sampling flowrate 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.

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

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 described in 9.8, and processed through the remaining analytical procedures along with the samples.

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

Analytical sensitivity (structures/ litre)	Limit of detection (structures/ litre)	Minimum number of grid openings (Litres of air sampled)						
		500	1 000	2 000	3 000	4 000	5 000	10 000
0.1	0.3	1 297	649	325	217	163	130	65
0.2	0.6	649	325	163	109	82	65	33
0.3	0.9	433	217	109	73	55	44	22
0.4	1.2	325	163	82	55	41	33	17
0.5	1.5	260	130	65	44	33	26	13
0.7	2.1	186	93	47	31	24	19	10
1.0	3.0	130	65	33	22	17	13	7
2.0	6.0	65	33	17	11	9	7	4
3.0	9.0	44	22	11	8	6	5	4
4.0	12	33	17	9	6	5	4	4
5.0	15	26	13	7	5	4	4	4
7.0	21	19	10	5	4	4	4	4
10	30	13	7	4	4	4	4	4

NOTE: In Table 1, it is assumed that one half of the sample collection filter is ashed, the volume of water used to disperse the ash is 40 ml, the volume of aqueous dispersion filtered is 34 ml, the active area of the analytical filter is 199 mm², and the TEM grid openings are square with a linear dimension of 85 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.

9 Procedure for analysis

9.1 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 PC and cellulose ester filters. The preparation method to be used shall be that described in either 9.4 or 9.5, depending on the type of membrane filter used for filtration of the aqueous dispersions. 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.

NOTE: 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 rinsed several times under a continuous flow of water. The glassware should then be rinsed at least twice using fibre-free water.

9.2 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.

9.3 Preparation of analytical filters

9.3.1 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 onto 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.

9.3.2 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 aluminium foil of thickness 0.015 mm to 0.02 mm, and bend the foil over the edges of the beaker so that it forms a tight seal. Perforate the foil in 10 to 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. 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.

NOTE: 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.

9.3.3 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 $\times$ 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 for a period of 5 min.

9.3.4 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 onto 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 PC filter or a 0.22 μm maximum pore size cellulose ester filter) onto 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.

9.3.5 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 fiber concentration. The volume of aqueous dispersion required to produce an analytical filter with a suitable particulate or fiber 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. Uniform deposits of particulate on the analytical filters cannot be assured 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, prepare only one analytical filter by filtration of the entire 40 ml dispersion.

NOTE: 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 fiber concentration is thought to be such that it is required to filter an aliquot of volume lower 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 five 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.

9.4 Preparation of TEM specimens from PC analytical filters

9.4.1 Selection of filter area for carbon-coating Use a cleaned microscope slide to support representative portions of polycarbonate (PC) filter during the carbon evaporation. Double-sided 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 a second cleaned glass microscope slide which is used as a cutting surface. Using a freshly-cleaned curved scalpel blade, cut the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. The scalpel blade and tweezers shall be washed and dried between the handling of each filter. Identify the filter portions by writing on the glass slide.

9.4.2 Carbon-coating of filter portions Place the glass slide holding the filter portions on the rotating-tilting device, approximately 10 cm to 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 PC filter will begin to curl, and crosslinking of the surface

will occur. This crosslinking 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; approximately 30 nm to 50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

9.4.3 Use of the Jaffe washer Place several pieces of lens tissue, as shown in Figure 3, on the stainless steel bridge, and fill the washer with chloroform or 1-methyl-2-pyrrolidone to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

9.4.4 Insertion of TEM specimens in the Jaffe washer Using a curved scalpel blade, cut three 3 mm square pieces of carbon-coated PC filter from the carbon-coated filter portion. Select three squares to represent the centre and the outer 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 onto the saturated lens tissue in the Jaffe washer. Place the three specimen grids from one sample on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for at least 8 h.

NOTE: It has been found that some PC 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 overheated during the carbon evaporation. It has been found that the problem of residual undissolved filter polymer can be overcome in several ways:

- a) a mixture of 20% 1,2-diaminoethane (ethylenediamine) and 80% 1-methyl-2-pyrrolidone, used in a Jaffe washer, completely dissolves PC filters in 15 min, even if the surface of the filter has been overheated. To use this solvent, place the grids directly on the stainless steel mesh of the Jaffe washer; do not use the lens paper. After a period of 15 min, transfer the stainless steel bridge into another Petri dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desired to retain water-soluble particle species on the TEM specimen grid, ethanol preferably be used instead of distilled water for the second wash;
- b) used in a Jaffe washer, 1-methyl-2-pyrrolidone has been found to be a more effective solvent than chloroform for PC filters. This solvent is more effective if the lens paper not used and grids are placed directly on the stainless steel mesh of the Jaffe washer. A dissolution period of 2 h to 6 h has been found to be satisfactory. After dissolution is complete, remove the stainless steel mesh from the Jaffe washer and allow the grids to dry. 1-Methyl-2-pyrrolidone evaporates very slowly. If it is required to dry the grids more rapidly, transfer the stainless steel bridge into another Petri dish, and add distilled water until the