

---

---

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

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



This document is a preview generated by EMS



**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2019

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Fax: +41 22 749 09 47  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

	Page
Foreword .....	v
Introduction .....	vi
<b>1 Scope .....</b>	<b>1</b>
<b>2 Normative references .....</b>	<b>1</b>
<b>3 Terms and definitions .....</b>	<b>1</b>
<b>4 Symbols and abbreviated terms .....</b>	<b>5</b>
<b>5 Type of sample .....</b>	<b>6</b>
<b>6 Range .....</b>	<b>6</b>
<b>7 Limit of detection .....</b>	<b>6</b>
<b>8 Principle .....</b>	<b>7</b>
<b>9 Reagents .....</b>	<b>7</b>
<b>10 Apparatus .....</b>	<b>8</b>
10.1 Air sampling — Equipment and consumable supplies .....	8
10.1.1 Filter cassette .....	8
10.1.2 Sampling pump .....	8
10.1.3 Stand .....	8
10.1.4 Personal sampling .....	8
10.1.5 Flowmeter .....	8
10.2 Specimen preparation laboratory .....	9
10.3 Equipment for analysis .....	9
10.3.1 Transmission electron microscope .....	9
10.3.2 Energy dispersive X-ray analyser .....	11
10.3.3 Plasma asher .....	11
10.3.4 Vacuum coating unit .....	11
10.3.5 Sputter coater .....	11
10.3.6 Solvent washer (Jaffe washer) .....	11
10.3.7 Condensation washer .....	12
10.3.8 Slide warmer or oven .....	13
10.3.9 Ultrasonic bath .....	13
10.3.10 Carbon grating replica .....	13
10.3.11 Calibration specimen grids for EDXA .....	13
10.3.12 Carbon rod sharpener .....	14
10.3.13 Disposable tip micropipettes .....	14
10.4 Consumable supplies .....	14
10.4.1 Copper or nickel electron microscope grids .....	14
10.4.2 Gold or nickel electron microscope grids .....	14
10.4.3 Carbon rod electrodes .....	14
10.4.4 Routine electron microscopy tools and supplies .....	14
10.4.5 Reference asbestos samples .....	14
10.4.6 Reference samples of mineral fibres other than asbestos .....	15
<b>11 Air sample collection .....</b>	<b>15</b>
<b>12 Procedure for analysis .....</b>	<b>16</b>
12.1 General .....	16
12.2 Cleaning of sample cassettes .....	16
12.3 Direct preparation of TEM specimens from polycarbonate filters .....	17
12.3.1 Selection of filter area for carbon coating .....	17
12.3.2 Carbon coating of filter portions .....	17
12.3.3 Preparation of the Jaffe washer .....	17
12.3.4 Placing of specimens in the Jaffe washer .....	17

12.4	Direct preparation of TEM specimens from cellulose ester filters .....	18
12.4.1	Selection of area of filter for preparation .....	18
12.4.2	Preparation of solution for collapsing cellulose ester filters .....	18
12.4.3	Filter collapsing procedure .....	18
12.4.4	Plasma etching of the filter surfaces .....	18
12.4.5	Carbon coating .....	18
12.4.6	Preparation of the Jaffe washer .....	18
12.4.7	Placing of specimens in the Jaffe washer .....	18
12.5	Criteria for acceptable TEM specimen grids .....	19
12.6	Procedure for structure counting by TEM .....	19
12.6.1	General .....	19
12.6.2	Measurement of mean opening area .....	20
12.6.3	TEM alignment and calibration procedures .....	20
12.6.4	Determination of criterion for termination of TEM examination .....	20
12.6.5	General procedure for structure counting and size analysis .....	21
12.6.6	Magnification requirements .....	23
12.7	Blank and quality control determinations .....	24
12.8	Calculation of results .....	24
<b>13</b>	<b>Performance characteristics .....</b>	<b>25</b>
13.1	General .....	25
13.2	Interferences and limitations of fibre identification .....	25
13.3	Precision and accuracy .....	25
13.3.1	Precision .....	25
13.3.2	Accuracy .....	26
13.3.3	Inter-laboratory and intra-laboratory analyses .....	26
13.4	Limit of detection .....	26
<b>14</b>	<b>Test report .....</b>	<b>27</b>
<b>Annex A (normative) Determination of operating conditions for plasma asher .....</b>		<b>30</b>
<b>Annex B (normative) Calibration procedures .....</b>		<b>31</b>
<b>Annex C (normative) Structure counting criteria .....</b>		<b>34</b>
<b>Annex D (normative) Fibre identification procedure .....</b>		<b>44</b>
<b>Annex E (normative) Determination of the concentration of asbestos fibres and bundles longer than 5 µm, and PCM equivalent asbestos fibres .....</b>		<b>61</b>
<b>Annex F (normative) Calculation of results .....</b>		<b>62</b>
<b>Annex G (informative) Strategies for collection of air samples .....</b>		<b>68</b>
<b>Annex H (informative) Methods for removal of gypsum fibres .....</b>		<b>69</b>
<b>Bibliography .....</b>		<b>70</b>

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

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

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

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

This second edition cancels and replaces the first edition (ISO 10312:1995), which has been technically revised. The main changes compared to the previous edition are as follows:

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

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

## Introduction

This document is applicable to the determination of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation of any atmosphere. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre sizes are the relevant parameters for evaluation of the inhalation hazards, a fibre counting technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos and therefore, there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This document is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. Airborne asbestos is often found as a mixture of single fibres and more complex, aggregated structures which may or may not be also aggregated with other particles. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if a sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis would become prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of, asbestos fibres is required. The method specified in this document is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. This document requires that a very detailed and logical procedure be used to reduce the subjective aspects of the measurement. The method of data recording specified in this document is designed to allow re-evaluation of the structure counting data as new medical evidence becomes available. All feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion onto a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. However, the procedures specified in this document are designed to minimize the disturbance of the collected particulate material, and the effect of those disturbances that do occur can be evaluated.

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

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

## 1 Scope

This document specifies a reference method using transmission electron microscopy for the determination of airborne asbestos fibres and structures in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation for asbestos structures in any atmosphere. The method allows determination of the type(s) of asbestos fibres present and also includes measurement of the lengths, widths and aspect ratios of the asbestos structures. The method cannot discriminate between individual fibres of asbestos and elongate fragments (cleavage fragments and acicular particles) from non-asbestos analogues of the same amphibole mineral<sup>[13]</sup>.

## 2 Normative references

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

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

## 3 Terms and definitions

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

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

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

### 3.1

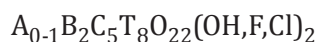
#### acicular

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

### 3.2

#### amphibole

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



where

- A = K, Na;
- B = Fe<sup>2+</sup>, Mn, Mg, Ca, Na;
- C = Al, Cr, Ti, Fe<sup>3+</sup>, Mg, Fe<sup>2+</sup>;
- T = Si, Al, Cr, Fe<sup>3+</sup>, Ti.