
**Nanotechnologies — Evaluation of
the antimicrobial performance of
textiles containing manufactured
nanomaterials**

*Nanotechnologies — Evaluation de la performance antimicrobienne
des textiles contenant des nanomatériaux manufacturés*

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Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Symbols and abbreviated terms	2
5 Characteristics of metal or metal oxide nanomaterials in TCMNMs	3
5.1 General	3
5.2 Physicochemical characteristics of metal or metal oxide nanomaterials	3
5.3 Characterization methods	4
6 Measurement of the released metal or metal oxide nanomaterials	4
6.1 Principle	4
6.2 Human perspiration solution preparation	4
6.2.1 General	4
6.2.2 Measurement method	4
6.3 Washing procedure	5
7 Determination of antimicrobial activities of TCMNMs	5
7.1 Principle	5
7.2 Antibacterial activity	5
7.3 Antifungal activity	5
7.4 Anti-odour property	6
8 Test report	6
Annex A (informative) Physical characterization techniques of nanomaterials in TCMNMs	9
Annex B (informative) Chemical characterization of nanomaterials in TCMNMs	10
Annex C (informative) Determination of antibacterial, antifungal, and anti-odour activity of TCMNMs	11
Bibliography	13

Foreword

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

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

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

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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.

This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

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

The utilization of nanotechnology in textile industry has presented novel functions such as antimicrobial activity, stain resistance, flame retardancy, mechanical strength enhancement, UV resistance, and wrinkle resistance into the conventional textiles without significant loss or change of the original properties. According to the nanodatabase website^[17] there are already over 400 textiles containing manufactured nanomaterials (TCMNMs), making them the second largest market among other nanoproducts.

The rapid and continued growth of TCMNMs is increasing the need to develop international standards specific for manufactured nanomaterials (MNMs) in textiles and testing processes guidelines. It is a dual need of industry and consumer.

TCMNMs can be classified into three groups based on how nanomaterials are integrated into the textiles including nanofinished, nanocomposite, and nanofibrous textiles^[1]:

- a) Nanofinished textiles: The textiles that the applied nanoscale property is added after the textile fabrication through post-manufacture treatments and coatings to create nanostructured surfaces on fibre media. Most nanotextiles on the consumer market belong to this category.
- b) Nanocomposite textiles: The textiles composed of fibres containing one or more nanostructured or nanoscale components produced by pre-manufacture integration of nanoscale properties into fibrous components.
- c) Nanofibrous textiles: The textiles made of nanofibres which have a nanoscale cross-sectional area and may or may not have a nanoscale length.

Natural and manufactured textile fibres can be treated with different nanomaterials and chemicals to provide enhanced antimicrobial properties. The antimicrobial activities of TCMNMs include activities against bacteria, fungal, viruses, and other microorganisms. Also, the antimicrobial activities can help to impart anti-odour property as the consequence of the reduced microbial activity. For antimicrobial TCMNMs, various metals, mainly silver and copper, and metal oxides such as copper oxide (CuO), titanium dioxide (TiO₂) and zinc oxide (ZnO) are normally used.

Several characteristics of MNMs have great impacts on their antimicrobial performance including size, shape, surface area, chemical composition, surface chemistry and surface charge. The size and shape of MNMs have important impacts on their antimicrobial property due to their association to their surface area. Generally, the antibacterial properties of nanoparticles are size-dependent. Smaller particles with higher surface area to volume ratio have more contact with either bacteria or fungi cells, or both, leading to improve either the bactericidal or fungicidal effectiveness, or both^[2]. Therefore, when they incorporate in textiles even at low concentrations they show noticeable antimicrobial activity compared to their micro- and macro scale counterparts.^{[3]-[5]} The shape of MNMs remarkably influences the rate of interaction and uptake by microbial cells. For instance, spherical-shaped of gold nanoparticles demonstrated higher cellular uptake than nanorod shaped particles^[7]. Surface charge of MNMs is another important characteristic that can be measured by Zeta potential method. The antimicrobial effect of MNMs is triggered by the electrostatic interaction between the positively charged MNMs and the negatively charged microbial cell membranes ultimately leading to cell damage and inhibition of their growth and reproduction. Surface chemistry of MNMs has an important effect on their antimicrobial activity. The presence of functional groups, capping agents or biomolecules on the surface of nanomaterials has also potential influence in their antibacterial activities. Surface functionalization of antimicrobial nanoparticles such as silver nanoparticles with bioactive molecules exhibited enhanced antibacterial activity compared to the bare ones^[8]. The above-mentioned inter-relationship highlights the important effect of physiochemical characteristics on antimicrobial performance of TCMNMs.

Currently, there are various antimicrobial TCMNMs products in the market such as underwear, shirts, socks, and bed sheets/covers. The antimicrobial mechanism of action of nanomaterials can generally be described as one of three models: oxidative stress induction, metal ion release, or non-oxidative mechanisms, which can occur simultaneously as well^[1]. The antimicrobial activity of

TCMNMs can decline significantly after several washing cycles and exposure to body sweat due to the possible release of incorporated nanomaterials and also the chemical action of sweat and laundering solution on the nanocompounds. Currently, there is no ISO document specific to TCMNM products. Therefore, the development of a standard to determine antimicrobial performance of TCMNMs subjected to washing process and body sweating can facilitate the trade and growth of market. It is worth mentioning that already published ISO standards are related to the assessment of antimicrobial properties of conventional textiles. Moreover, there is an ASTM standard document for detection and characterization of silver nanomaterials in textiles^[9]. However, these documents do not address the potential release of nanomaterials/nanostructure from TCMNNs following washing or sweating and their possible consequence on the antimicrobial activity of these textiles.

This document does not address nano-safety and environmental impact of the release of nanomaterials from TCMNMs into the air, water and to landfill. Data related to the release of nanomaterials from the fabrics under different conditions such as sweating, mechanical stresses (repetitive abrasion) during washing process in a laundry machine, are considered as essential information for understanding the potential releases to the environment.

Artificial sweat solution is an appropriate candidate to use as a material to resemble the human skin sweat to determine the amount of release of nanomaterials from TCMNMs to human body. For many TCMNMs applications, such as human clothes, there is a high possibility of skin contact and interaction with incorporated nanomaterials^[10]. In such condition, the involved interaction and release of the nanomaterial can also affect the antibacterial performance of TCMNMs.

Considering the effect of the release of nanomaterials from TCMNMs by washing process and human sweat, this document specifies the measurement methods of the released nanomaterials, the antimicrobial performance and the assessment method of TCMNMs. Further, from TCMNMs subjected to washing process and exposed to artificial human body sweat solution are specified.

Nanotechnologies — Evaluation of the antimicrobial performance of textiles containing manufactured nanomaterials

1 Scope

This document specifies the antimicrobial performance assessment method of textiles containing manufactured (metals/metal oxides) nanomaterials (TCMNMs). The textiles in this document include fabric, yarn and fibre in which manufactured nanomaterials are used during production or finishing process. Further, this document also specifies protocols to determine the quantity of nanomaterials released from textile following washing and/or exposure to artificial human body sweat.

This document only covers the antibacterial, antifungal, and the anti-odour performance assessment method of TCMNMs.

This document does not cover textiles that have therapeutic application as well as environment, health and safety (EHS) issues related to TCMNMs. Further, it does not cover the release of nanomaterials from TCMNMs as a result of aging, dry attrition and abrasion, although it is considered as an effective factor in releasing nanomaterials.

2 Normative references

The following referenced documents are indispensable for the application 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 105-E04, *Textiles — Tests for colour fastness — Part E04: Colour fastness to perspiration*

ISO 6330, *Textiles — Domestic washing and drying procedures for textile testing*

ISO 20743:2021, *Textiles — Determination of antibacterial activity of textile products*

ISO 13629-1, *Textiles — Determination of antifungal activity of textile products — Part 1: Luminescence method*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

EN 16711-1, *Textiles — Determination of metal content — Part 1: Determination of metals using microwave digestion*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6330, ISO/TS 80004-1 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
nanomaterial

material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of nano-object and nanostructured material.

[SOURCE: ISO/TS 80004-1:2015, 2.4, modified — Note 2 to entry has been deleted.]

3.2
textile

woven fabric, knitted fabric, etc., formed by the interlocking of fibres and yarns having certain cohesion and which is generally intended for clothing or furniture applications

Note 1 to entry: Textiles often include certain types of non-woven fabrics.

[SOURCE: ISO 16373-3:2014, 2.4]

3.3
antimicrobial activity

ability to kill/destroy/inactivate microorganisms, prevent their proliferation and/or prevent their pathogenic action

[SOURCE: ISO 18369-1:2017, 3.1.11.12]

3.4
antibacterial activity

activity of an antibacterial finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

[SOURCE: ISO 20743:2021, 3.4]

3.5
antifungal activity

activity to prevent or mitigate the growth of fungus, expressed as the difference of growth value in logarithm of *ATP* (3.6) between the control and test sample

[SOURCE: ISO 13629-1:2012, 3.6]

3.6
ATP

adenosine triphosphate, a multifunctional nucleotide present in living fungi

[SOURCE: ISO 13629-1:2012, 3.5]

3.7
washing procedure

cycle of the washing action including water supplying, washing, and repeated rinsing, spinning and water supplying and ended by spinning as predetermined on the washing machine

[SOURCE: ISO 6330:2012, 3.7]

4 Symbols and abbreviated terms

AAS	Atomic absorption spectroscopy
AFM	Atomic force microscopy
AES	Auger electron spectroscopy

ATP	Adenosine triphosphate
BET	Brunauer, Emmett, and Teller
ELS	Electrophoretic light scattering
FESEM	Field emission scanning electron microscopy
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
ICP-MS	Inductively coupled plasma-mass spectrometry
MNM	Manufactured nanomaterial
TCMNM	Textiles containing manufactured nanomaterial
TEM	Transmission electron microscopy
SAED	Selected area (electron) diffraction
XPS	X-ray photoelectron spectroscopy

5 Characteristics of metal or metal oxide nanomaterials in TCMNMs

5.1 General

As was mentioned earlier in the introduction section, knowledge about the physicochemical characteristics of nanomaterials used in TCMNMs is important, considering their noticeable effects on their antimicrobial performance. Subject to the stakeholder agreement and the specific application, these characteristics as shown in [Table 1](#) should be measured and reported.

A wide variety of analytical techniques are available for detection and characterization of nanomaterials in textiles. The selection of the appropriate techniques depends on capabilities, advantages and limitations of the techniques. Also, the cost and availability of the instrument need to be taken into account. There are no single techniques to both detect and characterize MNMs in textiles.

5.2 Physicochemical characteristics of metal or metal oxide nanomaterials

The commercially available techniques to measure the physicochemical characteristics of nanomaterials and definitions relevant to the characterization of them are available in ISO/TR 18196 and ISO/TS 80004-6, respectively. Also, the characteristics and measurement methods for powder or colloidal forms of silver nanoparticles applied as antibacterial agents are available in ISO/TS 20660.

These physicochemical characteristics include shape, size, surface charge, chemical composition, and surface chemistry of MNMs. [Table 1](#) summarizes the list of physicochemical characteristics and their measurement methods for TCMNMs.

Table 1 — List of physicochemical characteristics of metallic or metal oxides nanomaterials used in TCMNMs

Characteristic/property	Measurement methods
Particle size, shape, and size distribution	FESEM, TEM, SEM, AFM
Zeta potential	ELS
Surface area	BET
Surface chemistry	XPS, AES
Chemical composition	AAS, ICP-AES, ICP-MS
Phase identification	TEM/SAED

As mentioned before, nanomaterials utilized in textiles are either incorporated in the main fibre texture or applied as a coating onto the textiles by different methods. On the other hand, such fibres and textile fabrics made out of them can be further processed for different purposes. In some cases, there can be complexities for the characterization and detection of the nanomaterials used for antibacterial properties. This includes the possible elemental and chemical similarities of different chemical agents for various purposes (e.g. dyeing, printing) with those of the used nanomaterials. Therefore, to identify the latter from the former, care should be taken to choose a set of appropriate measurement techniques, since for such cases normally no single technique can be suitable to resolve the issue. In this respect, ASTM E3025-16^[15] also explores some of the physicochemical characteristics measurement methods and the relevant detection challenges of textiles containing silver nanomaterials which can be considered^[9].

5.3 Characterization methods

The brief description of the mentioned characterization methods of TCMNMs is given in [Annex A](#) and [Annex B](#). For chemical composition analysis, the sample shall be digested according to one of the procedures of acid digestion or microwave-assisted acid digestion presented in [Clause B.3](#). The goal of digestion is to completely decompose the solid matrix of TCMNMs to transfer the nanomaterials into the solution for the further determination step. The choice of the digestion method depends on the instrument availability and agreement between the concerned parties.

6 Measurement of the released metal or metal oxide nanomaterials

6.1 Principle

The nanomaterial released from textiles is measured during exposure to human perspiration and washing procedure as described in [6.2](#) and [6.3](#), respectively.

6.2 Human perspiration solution preparation

6.2.1 General

Artificial perspiration solution shall be used to simulate human perspiration. Since perspiration varies widely from one person to another, it is not possible to design a method with universal validity. Generally, fresh human perspiration is weakly acidic. However, micro-organisms cause the pH to become weakly alkaline (pH 7,5 to pH 8,5). Therefore, two different artificial alkaline (pH 8) and acidic sweat solutions (pH 5,5) as specified in ISO 105-E04 shall be utilized as the natural perspiration source. The preparation of artificial alkaline and acidic sweat solutions shall be made according to ISO 105-E04.

6.2.2 Measurement method

The amount of nanomaterials released from textiles is determined by measuring the concentration difference before and after they are being exposed to as-prepared artificial body sweat solution using the formula:

$$X = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where

- X is the amount of nanomaterials released from the textile sample;
- A_0 is the measured amount of nanomaterials ($\mu\text{g/l}$) in the textile sample solution before it is exposed to the artificial sweat solution;
- A_1 is the amount of nanomaterials ($\mu\text{g/l}$) in the textile sample after it has been exposed to the artificial sweat solution (to be reported as a percentage).

A_0 and A_1 shall be measured after subjecting the samples to acid digestion or microwave-assisted acid digestion as explained in [Annex B](#).

It should be mentioned that the measured release can be also due to the possible utilization of chemicals and or nano-sized particles as dyes. In such case, care must be taken to consider only the release of the nanomaterial(s) showing antimicrobial properties due to the limited available MNMs for such applications. Further, it is necessary that the producer or manufacturer should declare the type of MNMs used in textiles.

6.3 Washing procedure

In this method, the released nanomaterials utilized in TCMNMs are measured after several washing cycles by regular domestic washing procedure. Considering the wide varieties of textiles covered by this document, the selection of specific procedure of washing, drying and type of detergent provided in ISO 6330 shall be done based on the instructions provided by textile manufacturer. The released amount of nanomaterials during the washing procedure is calculated similarly according to the [Formula \(1\)](#). However, here, A_0 and A_1 are referred to the number of nanomaterials in textile sample pre- and post-washing process, respectively. A_0 and A_1 shall be measured after subjecting the samples to microwave or acid digestion as explained in [Annex B](#).

7 Determination of antimicrobial activities of TCMNMs

7.1 Principle

Antimicrobial activities of TCMNMs samples including antibacterial, antifungal and anti-odour shall be carried out on specimens, pre- and post-washing process and those before and after exposure to human sweat solution.

7.2 Antibacterial activity

The antibacterial activity of the TCMNMs shall be determined according to the ISO 20743:2021 standard method using Gram-positive *Staphylococcus aureus* and Gram-negative bacterium *Klebsiella pneumoniae* (AATCC 4352). A brief description of the method is given in [Clause C.1](#).

NOTE 1 Other bacteria can be used after appropriate validation.

NOTE 2 Refer to World Data Centre for Microorganisms (WDCM): <http://refs.wdcm.org/search.htm> [30].

7.3 Antifungal activity

The antifungal activity of TCMNM samples shall be determined according to ISO 13629-1. The test method determines the antifungal activity by measuring the intensity of luminescence produced by an enzymatic reaction (ATP method). A brief description of the method is given [Clause C.2](#).

The reference fungi to be used shall be selected from the following list:

- *Aspergillus niger*;
- *Penicillium citrinum*;

- *Cladosporium cladosporioides*;
- *Trichophyton mentagrophytes*.

NOTE 1 Other fungi can be used after appropriate validation.

NOTE 2 Refer to WDCM and its website: <http://refs.wdcm.org/search.htm>^[30].

7.4 Anti-odour property

Sweat secreted by axillary glands is odourless. The human axillary malodour is mainly produced by bacteria flora found on axillary skin dominated by genus of Gram-positive *Corynebacteria*^[11]. The antibacterial TCMNMs can reduce malodour by decreasing the number of *Corynebacteria* on axillary skin area. If the manufacturer claims that the product has the anti-odour property, this property of textile samples shall be determined according to ISO 20743 and carry out the test by using any strains of *Corynebacterium* such as *Corynebacterium xerosis*. A more detailed description is given in [Clause C.3](#).

8 Test report

The manufacturer or provider shall report the relevant general information and the measurement results of the fundamental characteristics of the TCMNMs. The test report shall contain the following information:

- a) a reference to this document (i.e. ISO/TS 23650:2021);
- b) general information of TCMNMs products such as product name, product application, batch number, lot number, manufacturing method, type of TCMNM, lab name and address;
- c) details of the measurement results of nanomaterial used such as size, size distribution, zeta potential, surface area, phase identification, chemical composition, and surface chemistry subject to the relevant stakeholders agreement;
- d) all details of antibacterial characterization test procedure such as name of the test bacteria, strain number, inoculation method, concentration inoculum, quantitative measurement method and antibacterial activity value (A), and measuring method according to ISO 20743;
- e) all details of antifungal characterization test procedure such as type of reference fungus, details of the fungus strain, spore concentration, inoculation method; growth value F , and antifungal activity value (A_a), measuring method according to ISO 13629-1 (if the antifungal property of TCMNMs is claimed by manufacturer);
- f) all details of antibacterial characterization test procedure such as name of the test bacteria, strain number, inoculation method, concentration inoculum, quantitative measurement method and antibacterial activity value (A), measuring method according to ISO 20743 (if the anti-odour property of TCMNMs is claimed by the manufacturer);
- g) all details regarding to the applied digestion method such as the type of digestion method, the applied acids and other reagents, and the time of digestion;
- h) all details relating to washing procedure such as the type of machine and washing procedure used, the drying procedure used, type of detergent used, number of applied washing cycles, measuring method according to ISO 6330;
- i) antimicrobial activities and the amount of nanomaterials of TCMNMs pre- and post-exposure to alkaline (pH 8) and acidic (pH 5,5) artificial sweat solutions, and the calculated released of nanomaterials (%) after exposure to artificial sweat;
- j) antimicrobial activities and the amount of nanomaterials of TCMNMs pre- and post-washing process, and the calculated released of nanomaterials (%) after washing procedure;
- k) any deviation from the test method;

- l) any unusual features observed;
m) the date of the test.

Table 2 gives an example of a possible report template.

Table 2 — Example of report template

Laboratory name		Laboratory address	
General			
Product name		Product application	
Batch number		Manufacturing method	
Lot number			
Fabric composition (% polyester, % cotton)		Fibre diameter	
Nanomaterial physicochemical characteristics and the applied characteristics method (optional, subject to the stakeholders agreement)			
Particle size, shape, and size distribution Applied method		Zeta potential Applied method	
Surface area Applied method		Phase identification Applied method	
Chemical composition Applied method		Surface chemistry Applied method	
Antibacterial characteristics			
Name of test bacteria		Strain number	
Inoculation method		Concentration inoculum	
Quantitative measurement method		Antibacterial activity value (A)	
Measurement method protocol according to ISO 20743		Testing date	
Antifungal characteristics			
Type of reference fungus		Spore concentration	
Details of the fungus strain		Inoculation method	
Growth value F		Antifungal activity value (A_a)	
Measurement method protocol according to ISO 13629-1		Testing date	
Anti-odour characteristics			
Name of test bacteria		Strain number	
Inoculation method		Concentration inoculum	
Quantitative measurement method		Antibacterial activity value (A)	
Measurement method protocol according to ISO 20743		Testing date	
Digestion method specification			
Applied digestion method ☐ Acid digestion ☐ Microwave-assisted acid digestion			
Applied acids and other reagents			
Time of digestion			
Washing procedure conditions			
Type of machine		Type of washing process	
Drying procedure		Type of detergent	
Number of applied washing cycles			

Table 2 (continued)

Laboratory name			Laboratory address		
Measurement method protocol according to ISO 6330			Testing date		
TCMNMs antimicrobial activities and amounts of nanomaterials pre- and post-exposure to alkaline (pH 8) artificial sweat					
Pre-exposure characteristics	Antibacterial activity value		Post-exposure characteristics	Antibacterial activity value	
	Antifungal activity value			Antifungal activity value	
	Anti-odour activity value			Anti-odour activity value	
	Measured amount of nanomaterials (µg/l)			Measured amount of nanomaterials (µg/l)	
					Release of nanomaterials (%)
TCMNMs antimicrobial activities and amounts of nanomaterials pre- and post-exposure to acidic (pH 5,5) artificial sweat					
Pre-exposure characteristics	Antibacterial activity value		Post-exposure characteristics	Antibacterial activity value	
	Antifungal activity value			Antifungal activity value	
	Anti-odour activity value			Anti-odour activity value	
	Measured amount of nanomaterials (µg/l)			Measured amount of nanomaterials (µg/l)	
					Release of nanomaterials (%)
TCMNM antimicrobial activities and amounts of nanomaterials pre- and post-washing process					
Pre-washing characteristics	Antibacterial activity value		Post-washing characteristics	Antibacterial activity value	
	Antifungal activity value			Antifungal activity value	
	Anti-odour activity value			Anti-odour activity value	
	Measured amount of nanomaterials (µg/l)			Measured amount of nanomaterials (µg/l)	
					Release of nanomaterials (%)

Annex A (informative)

Physical characterization techniques of nanomaterials in TCMNMs

A.1 Determination of size and size distribution

FESEM, AFM or TEM images can be used to evaluate the size of nanomaterials (see ISO/TS 12805, ISO 19749 and ISO 21363).

It is worth to mention that the quality and extent of information derived through these techniques, i.e. FESEM, TEM and AFM, depend on appropriate sample preparation. Further, it should be mentioned that the outcome of the results of MNMs size and size distribution measurements can be slightly different between different laboratories due to instrumental measurement sensitivity and the relevant limitations of each technique. To overcome this problem, care should be taken to use the same techniques with well-calibrated instruments.

To prepare the AFM sample, a single fibre from each textile sample is mounted onto a glass slide using adhesive tape, ensuring that the fibre was straight and strongly adhered to the slide. To obtain valid and repeatable results, measurements shall be repeated for three different individual fibres of three randomly selected areas of each fabric^[13].

Non-conductive textile specimens are generally coated with a layer of conductive materials such as carbon by high vacuum evaporation instrument. In addition to secondary electron detection mode, it is also recommended that the backscattered electron detection mode is applied to aid in detecting the nanomaterials in textiles as these metallic particles exhibits brighter contrast as compared to the textile matrix.

For TEM analysis, the specimen shall be prepared as a thin foil so that the electron beam can penetrate. TCMNMs samples can be directly cut into thin films (<100 nm) using an ultra-microtome with a diamond knife at a cryogenic condition (in liquid nitrogen), or first embedded in a resin and then cut into thin films using an ultramicrotome. Both cryogenic sectioning and resin sectioning require skill and patience of the user.

The size distribution of the nanomaterials observed by above mentioned techniques can be obtained through statistical method according to ISO 13322-1:2014.

A.2 Determination of zeta potential

Zeta potential is a method to measure the surface charge of nanomaterials. In this method, the surface charge is measured using the electrophoretic method, and the pH value shall be reported along with the surface charge. Zeta potential is not directly measured. It can be determined using proper theoretical models from experimentally determined parameters, such as electrophoretic mobility. Optical methods such as ELS have been widely used to determine electrophoretic mobility of particles or macromolecules in suspension or in solution. The details of zeta potential measurement using optical means can be found in ISO 13099-2.

A.3 Surface area

The surface area of nanomaterials shall be measured using a method based on BET nitrogen adsorption technique. BET analysis is a standard method to determine the specific surface area by measuring the amount of adsorbed gas. ISO 9277 specifies the measurement procedures for the overall specific external and internal surface areas of disperse or porous solids.

Annex B (informative)

Chemical characterization of nanomaterials in TCMNMs

B.1 Determination of chemical composition and quantification of MNMs

As highlighted in [Table 1](#), AAS, ICP-AES and ICP-MS methods can be used to determine the chemical composition and quantification of metallic components of MNMs in TCMNMs after acid digestion procedure.

ICP-AES and ICP-MS can express the type and content of elements of the measured samples (see ISO/TS 19590). For chemical composition analysis, the samples shall be digested according to the procedure of acid digestion or microwave-assisted acid digestion (see [Clause B.3](#)).

B.2 Determination of surface chemistry

XPS or AES is a quantitative spectroscopic surface chemical analysis technique used to estimate the empirical formula or elemental composition, chemical state and electronic state of the element on the surface (up to 10 nm) of a material. It also detects the contamination, if any, exists in the surface or the bulk of the sample.

B.3 Sample preparation by acid digestion or microwave-assisted acid digestion

Acid digestion methods can be conducted in either open system (under atmospheric pressure) or closed system (elevated temperature) using various mineral acids (e.g. HCl, HNO₃, HF, H₂SO₄), and other reagents such as hydrogen peroxide, potassium peroxide sulfate, and boric acid. Open acid digestion is performed in laboratory vessels heated by hot plate or heating mantle. In a closed acid digestion method, the mixture of samples, acids and reagents is heated by conventional or microwave ovens. If a microwave system is available, it is recommended to use it since it is a time-efficient and clean sample preparation method with optimal safety through continuous monitoring of the reaction parameters such as temperature and pressure. [Table B.1](#) summarized the specifications of open and closed acid digestion procedures.

The open system acid digestion method shall be done according to the EPA SW 846 Method 3050B^[28] based on the use of a strong acid digestion method which is applicable for almost all kind of metallic or metallic oxide nanomaterials. This method is not applicable for polyolefin (polypropylene) samples. Use EN 16711-1 to prepare textile samples for determination of metal content in a microwave-assisted acid digestion system.

Table B.1 — Specifications of open and closed acid digestion methods

Specification	Open acid digestion	Closed acid digestion
Maximum temperature	Limited to acid's boiling point	360 °C to 300 °C
Acid consumption	High	Low
Digestion quality	Frequently unsatisfactory	High
Sample loss	Loss of volatile elements	No loss
Contamination risk	Yes	No
Digestion time	2 h to 15 h	20 min to 60 min (microwave) 2 h to 5 h (conventional heating)

Annex C (informative)

Determination of antibacterial, antifungal, and anti-odour activity of TCMNMs

C.1 Determination of antibacterial activity

The qualitative test method for antibacterial activity of textile products is developed as ISO 20645. Several practical quantitative methods are presented in ISO 20743. The test methods are composed of two major steps of inoculation of bacteria and quantitative measurement of bacteria. Three different methods are specified for the inoculation of bacteria as below:

- a) absorption method — an evaluation method in which the test bacterial suspension is inoculated directly onto specimens;
- b) transfer method — an evaluation method in which test bacteria are placed on an agar plate and transferred onto specimens;
- c) printing method — an evaluation method in which test bacteria are placed on a filter and printed onto specimens.

Two methods of colony plate count and ATP luminescence methods are also specified for quantitative measurement of bacteria in ISO 20743. Therefore, there are six ways to combine the inoculation methods and quantitative measurements to accomplish this test for which the selection of antibacterial method depends on the agreement between the manufacturer and buyer. Results are expressed as a difference between logarithmic value of number of the bacteria in tested sample and in untreated control sample. The efficacy of antibacterial property of TCMNMs is given in [Table C.1](#).

Table C.1 — Efficacy of antibacterial property

Efficacy of antibacterial property	Antibacterial value A^a
Significant	$2 \leq A < 3$
Strong	$A \geq 3$
^a A is the difference between logarithmic value of number of the bacteria in tested sample and in untreated control sample.	

C.2 Determination of antifungal activities

The ISO 13629 series consists of two parts that differ in the quantitative methods for the determination of antifungal activities of textile products. ISO 13629-1 specifies an ATP luminescence method as a quantitative method and ISO 13629-2 specifies the plate count method. The user can select the most suitable evaluation method from the following methods before quantifying by any of two quantitative methods:

- a) absorption method — an evaluation method in which test fungi suspension is inoculated directly onto the specimens;
- b) transfer method — an evaluation method in which test fungi are placed on an agar plate and printed onto the specimens.