
**Wool — Determination of fibre
diameter — Projection microscope
method**

*Laine — Détermination du diamètre des fibres — Méthode du
microscope à projection*





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Foreword

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

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

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 38, *Textiles*, Subcommittee SC 23, *Fibres and yarns*.

This second edition cancels and replaces the first edition (ISO 137:1975), which has been technically revised.

This second edition to ISO 137 is based on the test method IWTO-8:2011, drawn up by the International Wool Textile Organization (IWTO).

Wool — Determination of fibre diameter — Projection microscope method

1 Scope

This International Standard specifies the procedure and the measurement conditions for the determination of the wool fibre diameter using a projection microscope.

The method is suitable for wool fibres in any form and also for other fibres of reasonably circular cross-section. (In the case of dyed, bleached or finished fibres, the diameter might be different from that of fibres not subjected to such treatments. The estimates of fibre diameter obtained at the various stages of processing one lot of wool will not necessarily be the same.)

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 139, *Textiles — Standard atmospheres for conditioning and testing*

ISO 1130:1975, *Textile fibres — Some methods of sampling for testing*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

mean diameter

average value of the projected width of either the wool fibre or another fibre of reasonably circular cross-section

3.2

total sample

sample intended to be representative of a large bulk of material, in the state in which it is sent to the laboratory

Note 1 to entry: The total sample is prepared according to the procedure specified in ISO 1130.

3.3

subsample

sample randomly drawn from and representative of the total sample, which has been suitably cleaned, dried and conditioned where appropriate

3.4

test specimen

part of a subsample which is tested at one time

4 Principle

Projection on a screen of the magnified images of the profiles of wool fibre snippets, and measurement of their width by means of a graduated scale. The operating technique ensures a random sampling of the fibres to be measured.

5 Apparatus

5.1 Projection microscope, comprising a light source, a light condenser, a stage which supports the slide carrying the fibres, an objective, an ocular and a circular screen.

5.1.1 Stage, movable in two directions at right angles by means of a sliding mechanism capable of successive displacements in 1,0 mm steps.

5.1.2 Objective and ocular, capable of providing 500X magnification.

5.1.3 Circular screen, equipped with a graduated scale capable of measuring the projected image of the fibre snippet on the screen in any orientation and position within the measuring area.

It is acceptable to mark a central circle having a diameter equal to one-quarter of the optical distance between the ocular and the centre of the screen. To ensure that any lens aberrations at the objective perimeter are avoided, all measurements must be made within this circle. However, some modern instruments contain much improved optics which ensure uniformity of magnification over the whole of the projected image. In the case of these instruments, no marked circle is needed and measurements can be made over the whole image area. In all cases where there is no marked circle on the screen, to ensure the integrity of the instrument's optics, the magnification should be checked over the whole projected image by using a certified graduated scale as described in [5.2](#).

NOTE A movable graduated scale made from transparent material and graduated on its underside in millimetres, as shown in [Figure 1](#), is suitable.

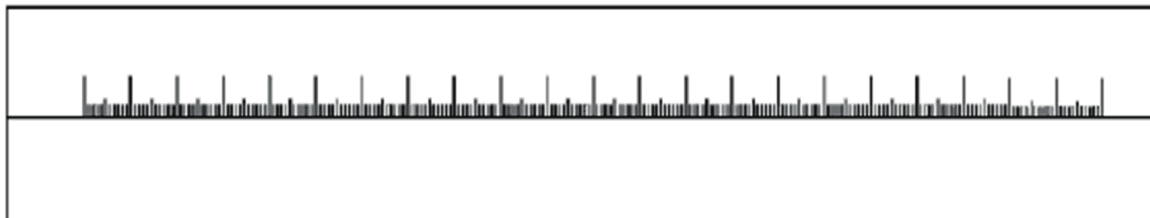


Figure 1 — Centre transparent graduated scale which slides between guides

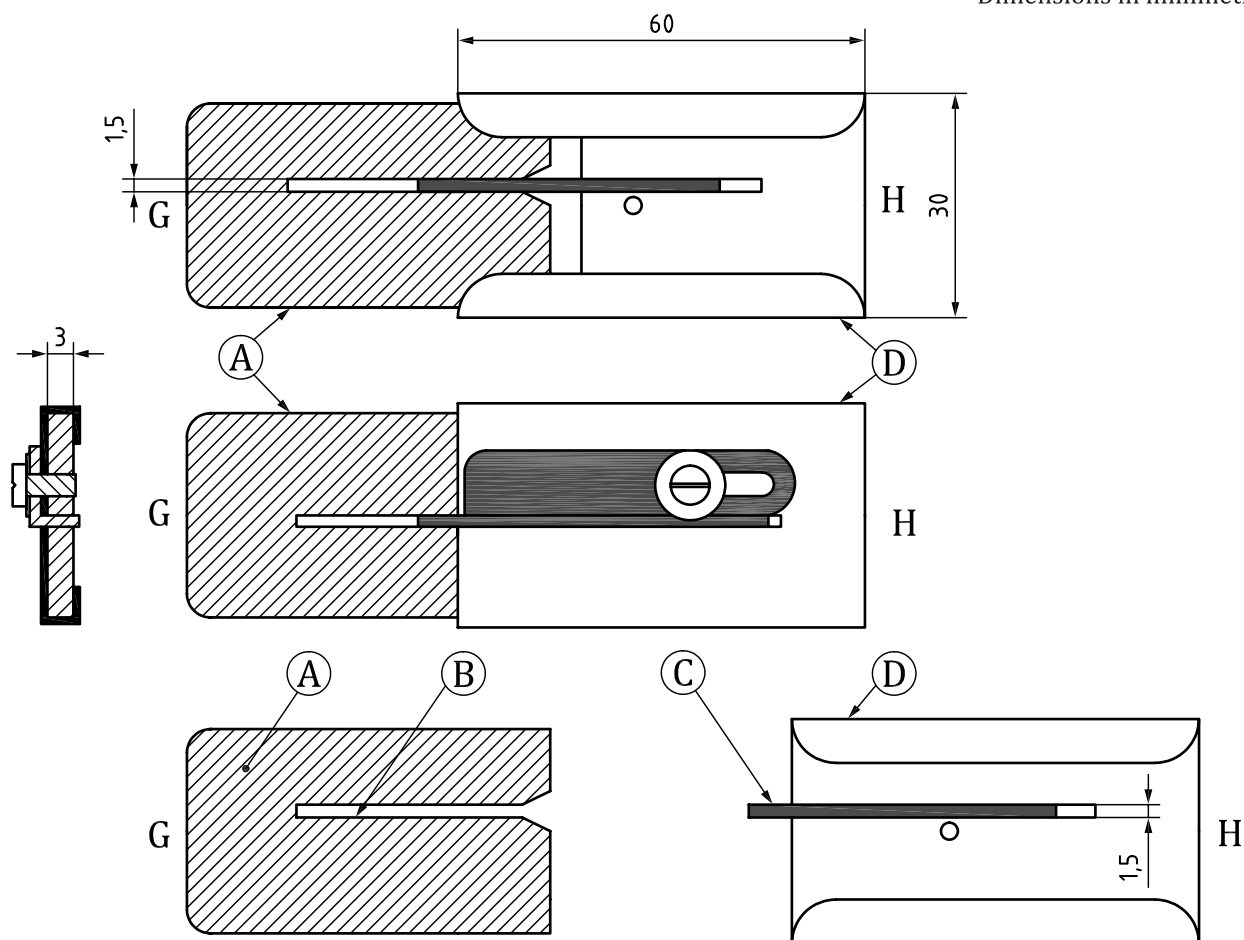
5.2 Micrometer graduated scale.

The projection microscope shall be calibrated periodically by means of a micrometer graduated scale (certified accurate), divided in hundredths of a millimetre and placed on the stage. One division of the micrometer (i.e. 0,01 mm), projected on the screen, shall cover exactly 5 mm of the graduated scale. The magnification is then equal to 500X.

5.3 Snippet cutters, suitable for cutting the fibres to a predetermined maximum length, capable of fulfilling the requirements of [6.3](#) regarding the cutting of the fibre pieces. The following apparatus ([5.3.1](#)) has been found suitable.

5.3.1 Fibre holder and pusher. These are shown in [Figures 2](#) and [3](#). The holder is a short piece of smooth steel (G) about 3 mm thick with a 1,5 mm slot into which slides the tongue of part H. The tongue of part H is fixed by a screw and may thus be adjusted to project different distances into the slot of G. The pusher consists of a steel stem with a short stop plate near its end; the stem has the same width as the slot, namely 1,5 mm. The stem of the pusher extends 0,8 mm beyond the stop plate.

Dimensions in millimetres



Key

- A steel plate
- B slot
- C steel tongue
- D guides

Figure 2 — Fibre microtome in which the wool sample is cut into pieces of predetermined length

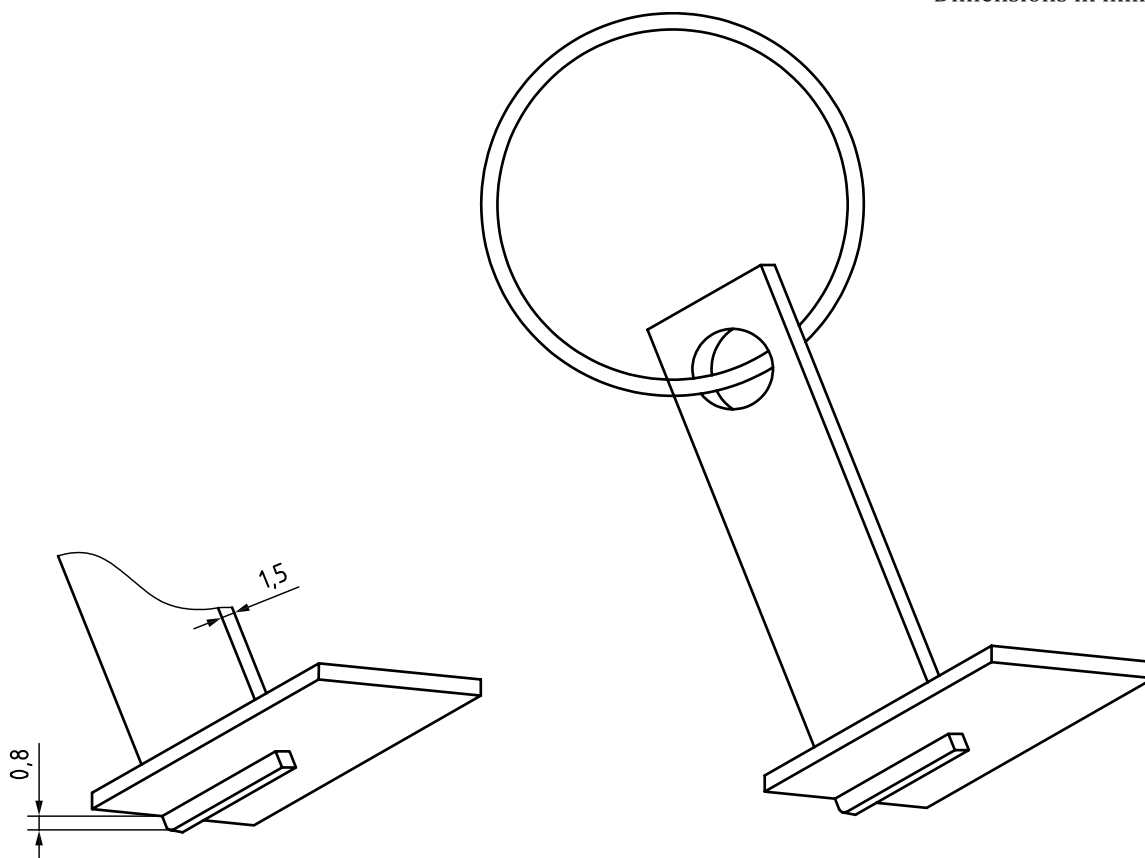


Figure 3 — A pusher by which a length of 0,8 mm of fibre can be pressed out

5.3.2 Conventional microtome.

Alternatively, a conventional microtome may be used if it is capable of fulfilling the requirements of [6.3](#) regarding the cutting of the fibre pieces.

5.4 Mounting medium, having the following properties:

- a) a refractive index between 1,43 and 1,53, at 20 °C;
- b) a suitable viscosity;
- c) zero water absorption;
- d) no effect on the diameter of the fibre.

Cedar wood oil and liquid paraffin are examples of suitable media. Anhydrous glycerine is not suitable.

5.5 Glass microscope slides, approximately 75 mm × 40 mm.

5.6 Cover glasses. Square or rectangular cover glass No. 1 (i.e. 0,13 mm to 0,17 mm thick) have been found suitable, as well as dimensions for the cover glass of 50 mm × 35 mm.

6 Sampling and preparation of the test specimens

6.1 Raw wool

6.1.1 Proceed in the following manner in accordance with ISO 1130:1975, 6.2.

Divide the mass of the total sample into roughly 40 zones and take a handful of fibres from each zone. Divide each handful into two (taking care to avoid breaking the fibres) and reject one-half, choosing the half to be rejected at random. If the fibres are parallel, make the division into two longitudinally, i.e. in a direction which avoids selection of fibres by their ends. Divide the retained half into two and again reject half at random. Continue in this way until 50 g of fibre remains.

6.1.2 Submit the reduced sample to a washing treatment consisting of two extractions in petroleum ether. Dry the sample and condition it in the standard atmosphere for conditioning given in ISO 139.

6.2 Slivers, rovings and yarns

6.2.1 From the total sample, which shall be as representative as possible of the bulk, take a sufficient quantity of material to fill the slot of the microtome to a sufficient depth. Long fibres are generally thick fibres, and consequently any manipulation resulting in selection of long fibres will lead to a greater diameter than mean diameter.

6.2.2 Condition the test specimen thus obtained in the standard atmosphere for conditioning given in ISO 139.

6.3 Cutting of snippets

6.3.1 Using a fibre holder and pusher

With the fibres in the slot G (as specified in [5.3](#)), insert the part H so that the tongue compresses the fibres firmly in the slot. To ensure satisfactory retention of the fibres, the length of the tongue should be suitably adjusted, then locked by means of the screw.

Then, using a sharp razor blade or scalpel, cut off the projecting tuft of fibres flush on both sides of the holder.

Insert the 0,8 mm pusher in the slot and slide it backwards and forwards so as to cause a fringe of fibres to project from the opposite side of the holder. With a razor blade, cut off this fringe of fibres flush with the surface of the holder, and mount as described in [6.4](#).

6.3.2 Using a microtome

With the fibres in the slot of the microtome, insert the tongued slide to retain them firmly. Using a sharp razor blade, cut off the surplus fibre from each side of the microtome plate.

Place the prepared plate on the ejector, having first ensured that the latter is returned to its lowest position, and lock it in place.

Eject fibres from the microtome plate of the required snippet cutting length (0,8 mm) by turning the micrometer wheel the required number of divisions. Using a sharp razor blade, cut off this protruding fringe of fibres flush with the surface of the plate.

6.4 Mounting of test specimen

The cut fibres are placed in a few drops of mounting medium ([5.4](#)) on a glass slide ([5.5](#)). The fibre pieces are then stirred well into the mounting medium, using a dissecting needle and employing a circular

motion so that a uniform distribution on the slide is obtained. Vigorous stirring should be avoided as this can introduce air bubbles which hinder the imaging of the fibres during measurement.

Sufficient of the mixture is then wiped cleanly away with a soft cotton cloth to ensure that no mixture is subsequently squeezed from under the cover glass. This avoids preferential removal of thin fibres.

A cover glass (5.6) is lowered onto the mixture by placing one edge in contact with the slide and gently lowering the opposite edge.

7 Test procedure

7.1 General

Each part of this technique is designed to ensure the following:

- random sample of the fibre snippets present on the slide is chosen for measurement;
- chance of measuring the same fibre snippet twice is negligibly small;
- operator has no free choice of the fibres to be measured.

If such a technique is not adopted, the danger of bias has been found to be very real.

7.2 Examination of the test specimen

Place the slide on the microscope stage, cover glass towards the objective. After the fibres have settled, the slide is examined in different fields. The distance between the centres of the fields should be greater than the length of snippets, otherwise the same snippet could be measured twice. For field centres separated by 1,0 mm, the probability of measuring the same snippet twice is very low for 0,8 mm snippet cutting length.

Begin the examination by moving the slide until a corner of the cover slip is focused. Then traverse the slide 1,0 mm (to B) then 1,0 mm in the transverse direction, thus bringing the first measurement area into view on the screen.

Measure the width of every fibre image lying within the measurement area, except the following which are not measured:

- a) images with more than half their width outside the central circle or images with widths not wholly within the boundary of the measurement screen for systems without central circles;
- b) images that end 2,5 cm from the point of measurement;
- c) images that cross another image within 2,5 cm from the point of measurement;
- d) images of fibres which are damaged.

Traverse the slide in 1,0 mm steps, using the sliding mechanism described in 5.1.1, and measure the fibres in each field as before. Continue traversing until the edge of the cover glass C is reached.

Cross-traverse the slide 1,0 mm distance and continue with a second traverse and then a third, etc. following the A B C D E F G etc. pattern in Figure 4 until the required number of measurements have been obtained. If necessary, prepare and measure extra slides.

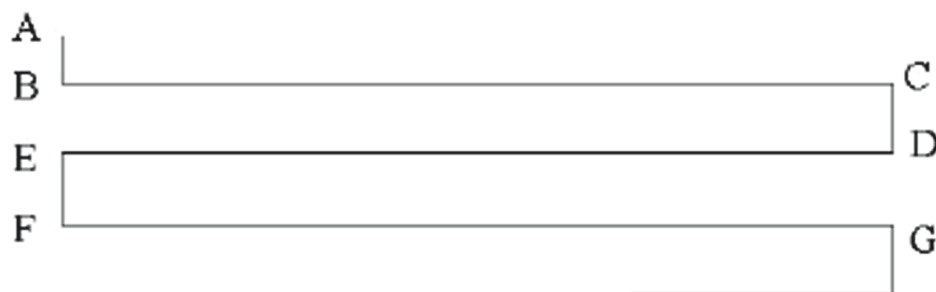


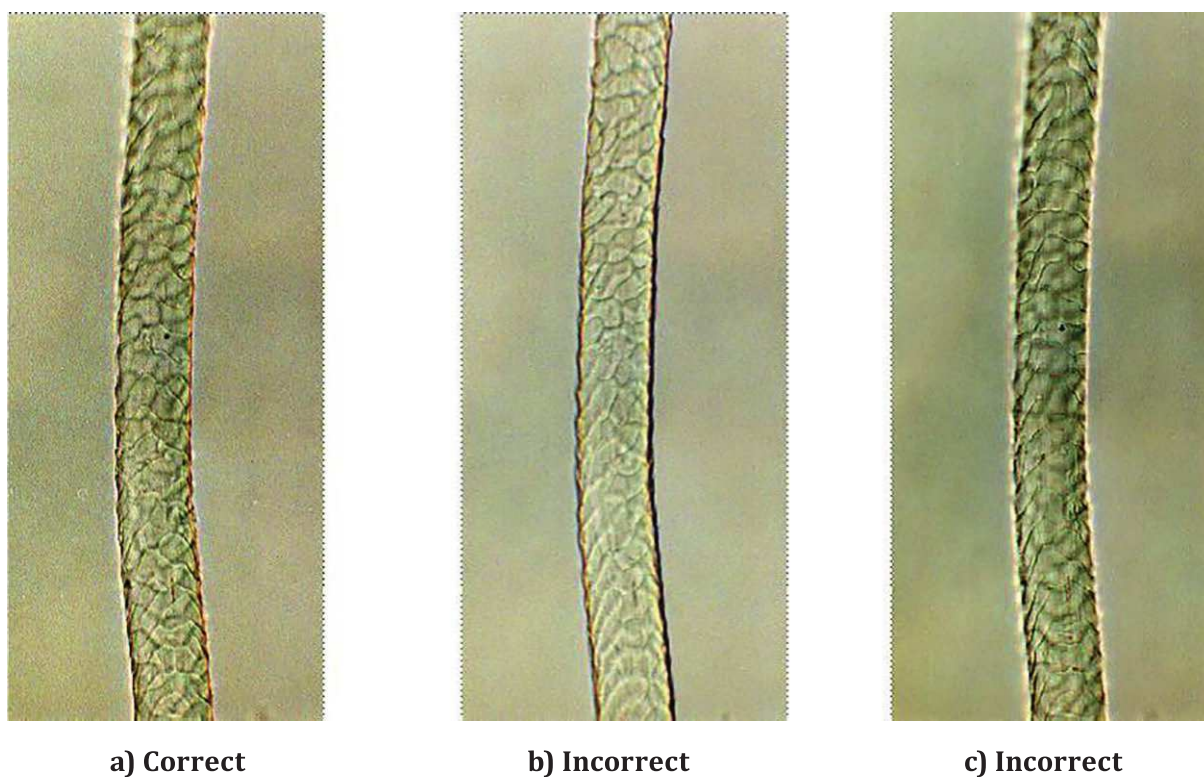
Figure 4 — Examination of the test specimen

7.3 Focusing

When the lens is too near the slide, a fibre edge shows a white border. When the lens is too far from the slide, a fibre edge shows a black border. These borders are called Becke lines.

When in focus, the fibre edge shows as a fine line without a border. However, it is not usual for both edges of a fibre image to be in focus together, since wool fibres are in general non-circular in cross-section.

When measuring a fibre whose edges are not in focus together, adjust the focusing so that one edge is in focus and the other shows a white line. Then measure the width from the edge that is in focus to the inside of the white line. [Figure 5](#) shows a fibre correctly and incorrectly focused.



a) Correct

b) Incorrect

c) Incorrect

Figure 5 — Correctly and incorrectly focused fibre

7.4 Measuring the width of a fibre image

Measure the width of a fibre image as follows.

Take the measurements at right angles to the lengthways direction of the image. If using a graduated scale or screen, move it with its length at right angles to the image until a centimetre division coincides with one edge of the image. Read off the width of the fibre image in millimetres.

Take the measurement between the points where the line of measurement crosses the image. Take the width as the distance between the extremities of the fibre image at this line even if the line happens to coincide with a fibre graduated scale or some other irregularity of the fibre. The stage should remain stationary during all measurements in a given field.

7.5 Recording of measurements

In general, the second edge of a focused image falls between 2 mm divisions of the graduated scale and is entered under the lower number of millimetres (N). In the subsequent calculation, all images recorded under N are assigned a width equal to $N + 0,5$ mm. However, sometimes the second edge of the image lies exactly on a millimetre division on the graduated scale. To avoid bias, these images shall be assigned alternately to this group (N) and to the lower millimetre group ($N-1$). This avoids assigning half an image to each group.

There are several methods for recording the results. Every time when one result is gotten in one group (N), the number of measurements (n_i) is recorded.

Other methods for recording the measurements, such as recording the individual results in computers and calculation of distribution parameters using standard software, are acceptable provided that the precision of an individual measurement is at least equal to $\pm 0,5$ mm and that snippet widths are assigned into group widths equivalent to diameters of 2 μm .

8 Measurement procedure

Each test specimen is measured using the technique described in [Clause 7](#). Where the density of snippets is such that 600 fibres cannot be measured, a further slide shall be prepared and further snippets measured until the required number of measurements have been obtained. Where more than one operator are used, the above procedures shall measure an approximately equal number of fibres so that the total of the fibres measured for the test specimen is 600.

It is recommended that at least two operators should carry out the measurements of the diameters of the fibre snippets on the slides.

9 Calculation and expression of results

The measurements in millimetres (lower limit $\pm 0,5$ mm) multiplied by 2 to give the diameter in micrometre for each class.

The mean value of fibre diameter (\bar{d}), the standard deviation of fibre diameter (S), the percentage coefficient of variation of fibre diameter (CV), are given by the following formulae:

$$\bar{d} = \frac{\sum(n_i \times d_i)}{\sum(n_i)} \quad (1)$$

$$S = \sqrt{\frac{\sum(n_i \times d_i^2) - \sum(n_i \times d_i)^2 / \sum(n_i)}{\sum(n_i) - 1}} \quad (2)$$

$$CV = \frac{S}{\bar{d}} \times 100 \% \quad (3)$$

where

\bar{d} is the mean value of fibre diameter (micrometres);

n_i is the number of measurements;

d_i is the measured value of fibre diameter (micrometres);

S is the standard deviation of fibre diameter (micrometres);

CV is the percentage coefficient of variation of fibre diameter.

Express the accuracy of the result by the confidence limits (see [Annexes A and B](#)).

10 Test report

The test report shall include the following information:

- a) the tests were conducted in accordance with this International Standard, i.e. ISO 137;
- b) the type, form and condition of the fibres tested;
- c) the total number of measurements (n);
- d) the mean value of fibre diameter (\bar{d});
- e) the standard deviation of fibre diameter (S), the percentage coefficient of variation of fibre diameter (CV) and the confidence limits;
- f) the laboratory that undertook measurement.

Annex A (informative)

Example of calculation

A.1 General

See [Table A.1](#).

Table A.1

Projected diameter mm	Recording marks of measurements	Number of measurements	Diameter		
		n_i	μm d_i	$n_i \times d_i$	$n_i \times d_i^2$
1					
2					
3					
4					
5		0	11	0	0
6	= = = =	27	13	351	4 563
7	= = = = = = =	48	15	720	10 800
8	= = = = = = = = = =	72	17	1 224	20 808
9	= = = = = = = = = = = =	78	19	1 482	28 158
10	= =	126	21	2 646	55 566
11	= = = = = = =	45	23	1 035	23 805
12	= = = = = = = = = = = = =	84	25	2 100	52 500
13	= = = = = = = =	51	27	1 377	37 179
14	= =	15	29	435	12 615
15	= = = =	27	31	837	25 947
16	= =	12	33	396	13 068
17	= =	12	35	420	14 700
18		3	37	111	4 107
19					
20					
Total		600		13 134	303 816

A.2 Mean value of fibre diameter (micrometres), \bar{d}

$$\bar{d} = \frac{\sum(n_i \times d_i)}{\sum(n_i)}$$

$$\bar{d} = \frac{13\,134}{600}$$

$$\bar{d} = 21,89$$

A.3 Standard deviation of fibre diameter (micrometres), S

$$S = \sqrt{\frac{\sum(n_i \times d_i^2) - \frac{(\sum(n_i \times d_i))^2}{\sum(n_i)}}{\sum(n_i) - 1}}$$

$$S = \sqrt{\frac{303\,816 - \frac{13\,134^2}{600}}{600 - 1}}$$

$$S = 5,22$$

A.4 Percentage coefficient of variation of fibre diameter (CV)

$$CV = \frac{S}{\bar{d}} \times 100\%$$

$$CV = \frac{5,22}{21,89} \times 100\%$$

$$CV = 23,85\%$$

Annex B (informative)

Accuracy of results and confidence limits for mean

Generally, only a small proportion of the fibres in the bulk can be measured and the sample mean is thus subject to the usual random sampling errors. The coefficient of variation of fibre diameter for unblended wool tops and worsted yarns is roughly 25 %, and the confidence limits when testing n fibres are given by Formula B.1:

$$\pm t \frac{S}{\sqrt{n}} \quad (\text{B.1})$$

where

S is standard deviation of fibre diameter (micrometres);

t is a factor which can be taken as equal to 1,96 for a confidence level of 95 %;

n is the total number of measurements.

The 95 % confidence limits, expressed as percentages of the mean value of the diameter, for different total numbers of fibres n , are given approximately in [Table B.1](#)

Table B.1 — Confidence limits

Confidence limits for a confidence level of 95 % as percentage of mean	Total number of measurements n
±1	2 500
±2	625
±3	278
±4	100

For blends and non-wool fibres, the coefficient of variation is different and different confidence limits should be applied.

Bibliography

- [1] IWTO-8:2011, *Method of determining fibre diameter distribution parameters and percentage of medullated fibres in wool and other animal fibres by the projection microscope*
- [2] GB/T 10685-2007, *Wool — Determination of fibre diameter — Projection microscope method*

