INTERNATIONAL STANDARD

ISO 21501-3

Second edition
2019-11

Determination of particle size distribution — Single particle light interaction methods —

Part 3: Light extinction liquid-borne particle counter

Détermination de la distribution granulométrique — Méthodes d'interaction lumineuse de particules uniques —

Partie 3: Compteur de particules en suspension dans un liquide par extinction de la lumière

Reference number
ISO 21501-3:2019(E)

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Published in Switzerland

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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).

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).

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.

This document was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

This second edition cancels and replaces the first edition (ISO 21501-3:2007), which has been technically revised. The main changes from the previous edition are as follows:

— Clause 4 for “Principle” and Clause 5 for “Basic configuration” have been added;

— “size calibration” and “verification of size setting” have been combined as “size setting error” in the requirements (Clause 6);

— “Test report” (3.10 in the previous edition) has been changed to 6.9 on “Reporting of test and calibration results”;

— information about uncertainties has been enriched and is now the subject of Annex B.

A list of all parts in the ISO 21501 series can be found on the ISO website.

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

Monitoring particle contamination levels is required in various fields, e.g. in the electronic industry, in the pharmaceutical industry, in the manufacturing of precision machines and in medical operations. Particle counters are useful instruments for monitoring particle contamination in liquid. The purpose of this document is to provide a calibration procedure and verification method for particle counters, so as to minimize the inaccuracy in the measurement result by a counter, as well as the differences in the results measured by different instruments.

Determination of particle size distribution — Single particle light interaction methods —

Part 3: Light extinction liquid-borne particle counter

1 Scope

This document describes a calibration and verification method for a light extinction liquid-borne particle counter (LELPC), which is used to measure the size and particle number concentration of particles suspended in liquid. The light extinction method described in this document is based on single particle measurements. The typical size range of particles measured by this method is between 1 µm and 100 µm in particle size.

The method is applicable to instruments used for the evaluation of the cleanliness of pharmaceutical products (e.g. injections, water for injections, infusions), as well as the measurement of number and size distribution of particles in various liquids.

The following are within the scope of this document:

— size setting error;

— counting efficiency;

— size resolution;

— maximum particle number concentration;

— sampling flow rate error;

— sampling time error;

— sampling volume error;

— calibration interval;

— reporting results from test and calibration.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
calibration particles
monodisperse spherical particles with a known mean particle size, e.g. polystyrene latex (PSL) particles, where the certified size is traceable to the International System of Units (SI), a relative standard uncertainty of the certified size is equal to or less than 2,5 %, a refractive index that is approximately 1,59 at the wavelength of 589 nm (sodium D line)

Note 1 to entry: For spherical particles, the particle size is equal to the diameter.

3.2
counting efficiency
ratio of the particle number concentration measured by a *light extinction liquid-borne particle counter* (3.3) of a *certified reference material* (3.7) for particle number concentration to the certified value of the CRM

3.3
LELPC
light extinction liquid-borne particle counter
instrument that measures liquid-borne particle numbers by counting the pulses as the particles pass through the sensing volume, as well as particle size by the attenuation of light

Note 1 to entry: The optical particle size measured by the LELPC is the light extinction equivalent particle size and not the geometrical size.

3.4
PHA
pulse height analyser
instrument that analyses the distribution of pulse heights

3.5
size resolution
measure of the ability of an instrument to distinguish between particles of different sizes

3.6
coincidence loss
reduction of particle count caused by multiple particles passing simultaneously through the sensing volume and/or by the finite processing time of the electronic system

3.7
CRM
certified reference material
<particle number concentration> particle suspension, typically polystyrene latex particles suspended in pure water, sufficiently homogeneous and stable, characterized for the mean particle size and number concentration by a metrologically valid procedure, accompanied by a reference material certificate that provides the associated uncertainties for the traceable values, and a statement of metrological traceability

Note 1 to entry: If no CRM standards are available, the use of a particle suspension characterised with corresponding uncertainties for particle size and concentration is sufficient.

3.8
MPE
maximum permissible error
limit of error
extreme value of measurement error, with respect to a known reference quantity value, permitted by specifications for a given measurement, measuring instrument, or measuring system

Note 1 to entry: This document uses decimal numbers for the requirements to MPEs to avoid confusions that may arise when relative uncertainties of test results are reported in percent figures.

4 Principle

The measurement principle of the LELPC is based on detection of light extinction by a particle when the particle passes through an incident light beam.

The particle size is determined from the attenuation of light, and the number of particles from the number of light extinction pulses by individual particles.

More specifically, a sample liquid is drawn from the inlet of the LELPC at a constant flow rate, and introduced to the sensing volume of the LELPC where a light beam is irradiated. When a particle suspended in the sample liquid passes through the light beam, it attenuates the light, occurring a light extinction pulse. The light extinction pulse is detected by a photo detector, and converted to an electrical pulse. The electrical pulse height is proportional to the attenuation of light, and depends on the optical system design, the electronic components used, and the light source. The attenuation of light is dependent on the size, refractive index, and shape of the particle. In order to establish a relationship between the electrical pulse height and the particle size, calibration of each LELPC with use of particles having a well-defined size, refractive index, and shape is required.

5 Basic configuration

An LELPC is composed typically of a light source, a sample liquid supply/suction system, a sensing volume, a photoelectric conversion device, a pulse height analyser, and a display (see Figure 1). Some LELPCs do not contain a sample liquid supply/suction system and/or a display.

To make the particle size calibration possible, the LELPC should be constructed so that pulse height distributions for calibration particles can be measured.

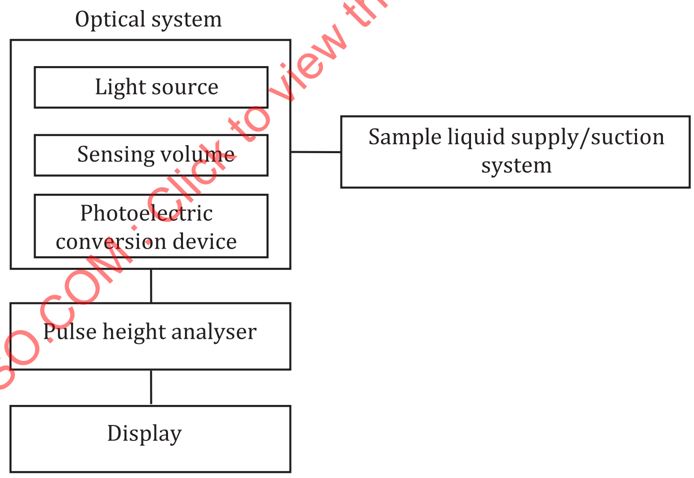


Figure 1 — Example of basic configuration of LELPC

6 Requirements

6.1 Size setting error

The MPE for size setting in the minimum detectable particle size and other sizes specified by the manufacturer of an LELPC is 0,10 (corresponding to 10 % of the specified size).

Size setting shall be conducted before the LELPC is shipped from the manufacturer, and when the size setting error is found not fulfilled in a periodic calibration.

A recommended procedure for size setting is described in 7.1.2. If other methods are used, their uncertainty shall be evaluated and described.

6.2 Counting efficiency

The counting efficiency shall be within 0,80 to 1,20 [corresponding to (100 ± 20) %] when the test is carried out by the method described in 7.2.

6.3 Size resolution

The size resolution shall be less than or equal to 0,10 (corresponding to 10 %) when the test is carried out by the method described in 7.3.

6.4 Maximum particle number concentration

The maximum measurable particle number concentration shall be specified by the manufacturer. The coincidence loss at the maximum particle number concentration of an LELPC shall be less than or equal to 0,1 (corresponding to 10 %).

NOTE The probability of occurrence of coincidence loss increases with increasing particle number concentration.

6.5 Sampling flow rate error

The MPE of the sampling flow rate shall be specified by the manufacturer. The user shall check that the sampling flow rate is within the range specified by the manufacturer.

If the LELPC does not have a flow rate control system this subclause does not apply, however the manufacturer shall specify the allowable flow rate range of the LELPC.

6.6 Sampling time error

The MPE in the duration of sampling time shall be 0,01 (corresponding to 1 %) of the preset value.

This subclause does not apply when the LELPC is not equipped with a sampling system.

This subclause does not apply when the LELPC is equipped with a volumetric sampling system.

6.7 Sampling volume error

The MPE of sampling volume shall be 0,05 (corresponding to 5 %) of the preset value.

This subclause does not apply when the LELPC is not equipped with a volumetric sampling system.

6.8 Calibration interval

The calibration of the LELPC should be conducted at an interval equal to or shorter than one year. The requirements should be met during the calibration interval.

6.9 Reporting of test and calibration results

The report shall contain at least the following information:

a) date of test/calibration;

b) test/calibration particles used;

c) results for the parameters:

   1) size setting error;

   2) counting efficiency;

3) sampling flow rate error;

4) size resolution (with the particle size used);

d) threshold voltage values or channel of the built-in PHA corresponding to the size settings;

e) reference of the test/calibration method used (i.e. ISO 21501-3).

f) report/certificate identification, test/calibration location, title and identification of test/calibration provider including signature and date;

g) identification of customer and device under test, including how output was obtained for counting efficiency (e.g. analogue, display or digital output).

A calibration certificate shall furthermore include:

h) identification and — if possible — statement of metrological traceability of all reference equipment and calibration particles used;

i) relevant environmental conditions (e.g. temperature, air pressure and humidity) under which the calibration was performed;

j) a stated uncertainty for each result for the parameters 1 to 2 with reference to the calculation method (e.g. ISO/IEC Guide 98-3) — Annex B gives a recommended procedure for evaluating the uncertainty of the results of the performance tests.

NOTE Calibration certificates issued by ISO/IEC 17025 accredited laboratories and covering all results for the parameters 1 to 2 are considered to comply with the requirements above.

7 Test and calibration procedures

7.1 Size setting

7.1.1 Evaluation of size setting error

Calculate the size setting error ε according to Formula (1).

$$\varepsilon = \frac{x_s - x_c}{x_c} \tag{1}$$

where

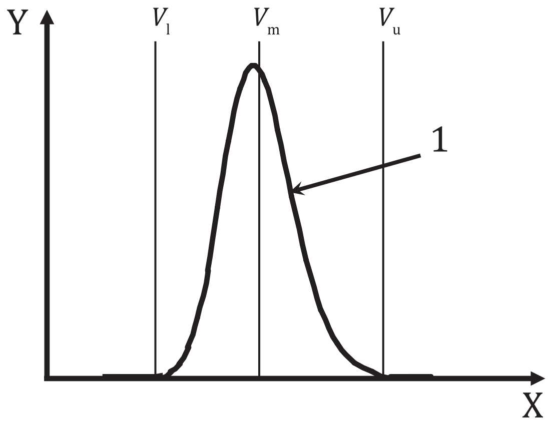
x_c is the certified size of the particles of the suspension of the certified reference material;

x_s is the particle size corresponding to 50 % counts of C_C (see 7.1.2 for the meaning of C_C).

7.1.2 Procedure of size setting

By use of a PHA connected to the output terminal for signal pulses of the LELPC, or by use of a built-in PHA if one is contained as a part of the LELPC, obtain a pulse height distribution for a sample liquid in which calibration particles are suspended. Let V_l and V_u denote the lower and upper voltage limits, respectively, of the range of pulse heights for the calibration particles (see Figure 2). The median voltage V_m of the pulse height distribution in the range from V_l to V_u, shall be calculated, and is assigned to the certified size of the calibration particles, x_c.

When a built-in PHA is used, the abscissa of the pulse height distribution may be given in channel number instead of voltage. In this case, the term "voltage" above and in relevant descriptions below should be interpreted as channel number of the PHA.



Key

X pulse height voltage

Y frequency

1 pulse height distribution

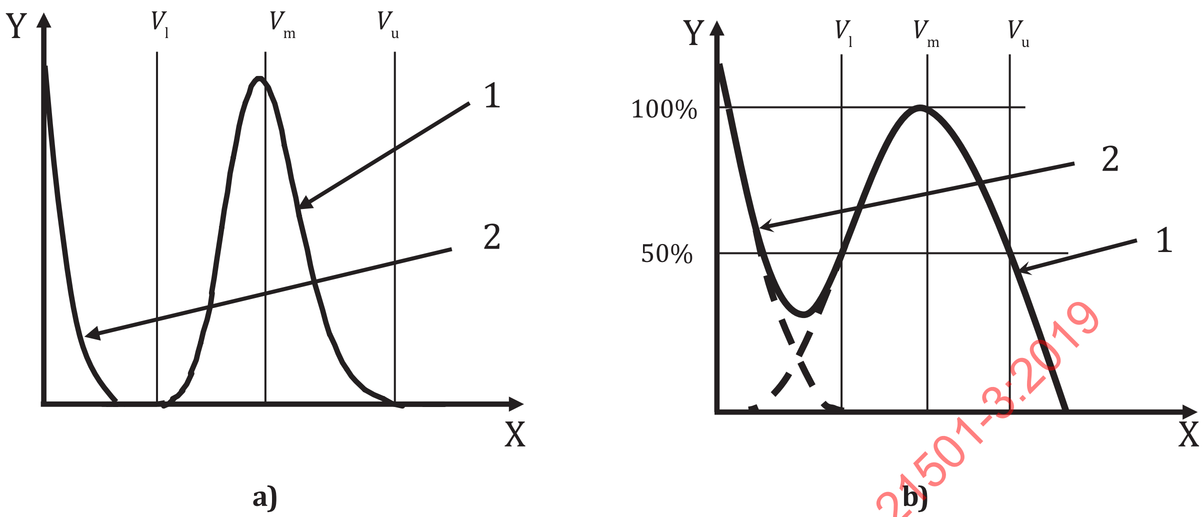
V_l lower voltage limit

V_m median voltage

V_u upper voltage limit

Figure 2 — Pulse height distribution for the sample liquid

If a noise distribution is observed in the pulse height distribution, and if it is separated distinctly from the main peak corresponding to the calibration particles, the voltages V_l and V_u shall be chosen so that the range (V_l, V_u) encompasses only the main peak [see Figure 3 a)]. If the noise distribution overlaps with the main peak, V_l and V_u shall be chosen so that the range (V_l, V_u) corresponds to the full width at half maximum of the main peak [see Figure 3 b)]. The latter way of determining V_l and V_u is allowed only when the height of the valley between the noise distribution and the main peak is at most half the main peak height.



Key

X pulse height voltage

Y frequency

1 pulse height distribution for calibration particles

2 noise distribution (false particles, small particles and/or optical or electrical noises)

V_l lower voltage limit

V_m median voltage

V_u upper voltage limit

Figure 3 — Pulse height distribution for the sample liquid when noise exists

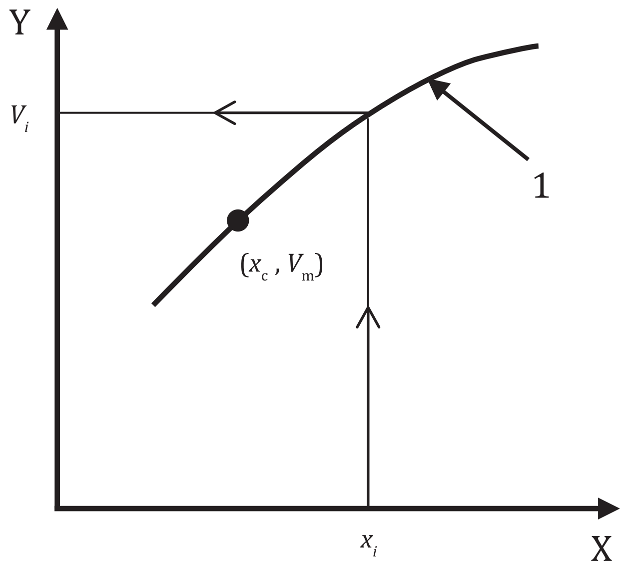
By use of the data pair (x_c, V_m) obtained in this way, or multiple data pairs (x_{cj}, V_{mj}) (j = 1, 2, ...) obtained similarly for multiple calibration particles, determine the voltage values V_i (i = 1, 2, ...) that correspond to the size settings (or threshold sizes) x_i given as specifications of the LELPC (see Figure 4). In this determination, a theoretical response curve based on Mie theory may be used to calculate V_i from experimentally observed V_m.

Let V_{ti} denote the adjustable threshold voltage corresponding to x_i. For all the size settings x_i, adjust the value of V_{ti} to V_i.

NOTE 1 The response curve can be calculated according to the Mie theory when the parameter set defining the optical system of the LELPC is available. If the parameter set of the optical system is not available, the response curve in the vicinity of x_i can still be empirically determined by fitting a simple function, e.g. a quadratic or cubic polynomial, to multiple data pairs (x_{cj}, V_{mj}) obtained for x_{cj} on either side of x_i.

NOTE 2 The detailed procedure for determining V_i can vary depending on the model of the LELPC

NOTE 3 V_{ti} can be the set voltage of an electric comparator used in the LELPC, or if a built-in PHA is used, it can be the threshold channel of the built-in PHA which is intended to be assigned to x_i. For the sake of simplicity in description, it is assumed that electric comparators are employed in the LELPC, unless otherwise stated.



Key

X particle size

Y pulse height voltage

1 response curve

x_c certified size of the calibration particles

V_m median voltage corresponding to x_c

x_i size setting specified for the LELPC

V_i voltage corresponding to x_i

Figure 4 — Size calibration

To calculate the size setting error of the LELPC, use the CRM.

Set the LELPC to count in the cumulative mode, collect counts, C_C, at a setting greater than or equal to half particle size of the CRM, and a particle size of 50 % counts of C_C. The size setting error ε is calculated as in Formula (1).

7.2 Evaluation of counting efficiency

To evaluate the counting efficiency of the LELPC, use the CRM.

Set the LELPC to count in the cumulative mode, collect counts at a setting greater than or equal to half particle size of the CRM.

Calculate the counting efficiency by means of Formula (2):

$$\eta = \frac{C_L}{C_R} \quad (2)$$

where

η is the counting efficiency;

C_L is the observed particle number concentration by the LELPC;

C_R is the particle number concentration of the CRM.

7.3 Evaluation of size resolution

Calculate the size resolution of the LELPC, R, by Formula (3) (see also Annex A).

$$R = \frac{\sqrt{\sigma^2 - \sigma_c^2}}{x_c} \qquad (3)$$

where

R is the size resolution;

σ is the apparent standard deviation of the size distribution of the calibration particles observed by the LELPC;

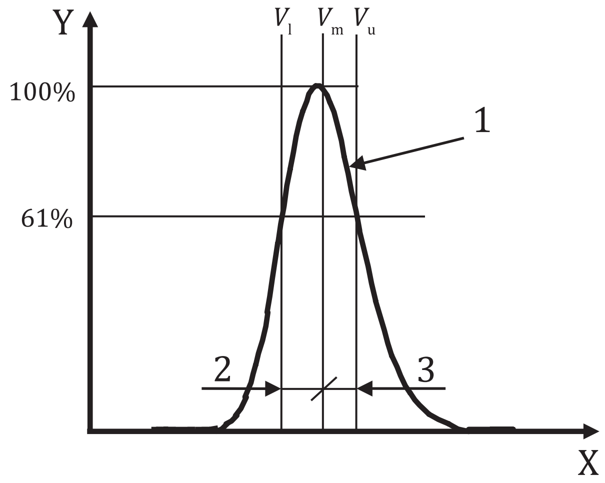
σ_c is the standard deviation of the size distribution of the calibration particles provided by the manufacturer of the calibration particles;

x_c is the certified average size of the particles of the CRM.

NOTE Due to the uncertainties in determining σ and σ_c, σ^2 can, in some cases, be smaller than σ_c^2. In such cases, the value of R is regarded as 0.

The CRM should be used for this test. The particle size recommended by the manufacturer of the LELPC should be used. The standard deviation of the calibration particles, σ_c, should be known. It is recommended to determine the median voltage (or channel) V_m of the pulse height distribution for the calibration particles, as shown in Figure 5, in accordance with the method given in 7.1.2.

Determine the lower and upper voltage limits, V_l and V_u, which correspond to 61 % of the peak height in the pulse height distribution. Using the calibration curve, determine the particle sizes x_l and x_u corresponding respectively to V_l and V_u. Calculate the absolute value of the differences, $|x_l - x_c|$ and $|x_u - x_c|$, where x_c is the certified size of the calibration particles. Let the apparent standard deviation, σ, be equal to the larger one of $|x_l - x_c|$ and $|x_u - x_c|$.



Key

X pulse height voltage (or channel)
Y frequency
1 pulse height distribution for the calibration particles
2 lower side resolution
3 upper side resolution
V_l lower voltage limit
V_m median voltage
V_u upper voltage limit

Figure 5 — Verification of size resolution

7.4 Estimation of coincidence loss at the maximum particle number concentration

The coincidence loss is determined by the flow rate, the time required for particles to pass through the sensing volume and the electrical signal processing time. These values are determined by the design of the LELPC. Coincidence loss is calculated as in Formula (4).

$$L = 1 - \exp\left(-q \cdot t_{total} \cdot C_{max}\right) \quad (4)$$

where

L is the coincidence loss at the maximum particle number concentration;

q is the flow rate;

t_{total} is the sum of the time for a particle to pass through the sensing volume and electrical processing time;

C_{max} is the maximum particle number concentration.

7.5 Evaluation of sampling flow rate error

Obtain a flow rate by the sampling volume (see 7.7) and the sampling time (see 7.6), or use a calibrated flow meter. Calculate the error in the sampling flow rate, ε_q, by Formula (5).

$$\varepsilon_q = \frac{q_m - q_s}{q_s} \tag{5}$$

where

ε_q is the sampling flow rate error;

q_s is the sampling flow rate specified by the manufacturer;

q_m is the measured sampling flow rate.

If the LELPC does not have a sampling function, this subclause does not apply.

7.6 Evaluation of sampling time error

Sampling time is the time during which the LELPC measures a sample (from the beginning of counting to the end of counting). Calculate the error in the sampling time, ε_t, by Formula (6).

$$\varepsilon_t = \frac{t_m - t_0}{t_0} \tag{6}$$

where

ε_t is the sampling time error;

t_0 is the sampling time preset to the LELPC;

t_m is the measured sampling time.

Calibrated instruments should be used for sampling time measurement.

7.7 Evaluation of sampling volume error

Measure the sampling volume by weighing the pure water with the balance and converting to volume, or measure the volume by means of a calibrated graduated cylinder.

If the LELPC does not have a sampling function, this subclause does not apply.

Annex A
(informative)

Size resolution

Size resolution denotes one standard deviation of the measured size distribution of monodisperse calibration particles, expressed as the mean size of the monodisperse calibration particles.

If the distribution of calibration particles is assumed to be the Gaussian distribution,

$$f(x)=\frac{1}{\sqrt{2\pi}\sigma}\exp\left\{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2\right\} \quad \text{(A.1)}$$

where

$f(x)$ is the Gaussian function;

x is the particle size;

μ is the mean value;

σ is the standard deviation.

when $(x-\mu)=\pm\sigma$, the ratio of density to the maximum density is $\exp\left(-\frac{1}{2}\right)\approx 0{,}61$. This is the basis for the use of 61 % in the determination of size resolution.

Annex B
(informative)

Procedure for evaluating the uncertainties of the results of the performance tests

B.1 Basics on measurement uncertainty

In this annex, a recommended procedure is described for evaluating the uncertainties of the results of the tests specified in 7.1 and 7.2 (see Note 1). This procedure follows ISO/IEC Guide 98-3, which is briefly summarized as follows.

Step 1) Identify the relationship between the measurand, y, and the input quantities, x_i (i = 1, 2, ..., N):

$$y = f(x_1, x_2, ..., x_N) \tag{B.1}$$

This functional relationship is called the mathematical model of measurement (see Notes 2, 3).

Step 2) Evaluate the standard uncertainty $u(x_i)$ of the input quantity x_i either by Type A or Type B evaluation of uncertainty (see Notes 4, 5).

Step 3) Combine the standard uncertainties of all x_i values to obtain the combined standard uncertainty of the measurement result, $u_c(y)$, according to the following 'law of propagation of uncertainty, (see Notes 6, 7).

$$u_c(y) = \sqrt{\sum_{i=1}^{N}\left(\frac{\partial f}{\partial x_i}u(x_i)\right)^2} \tag{B.2}$$

Step 4) When necessary, the expanded uncertainty U is calculated according to Formula (B.3):

$$U = k \times u_c(y) \tag{B.3}$$

where k is the coverage factor. In this standard, k = 2 is consistently used for simplicity (see Note 8).

NOTE 1 The uncertainty components considered in this annex are those relevant to the tests specified in the main body of this document. These components are considered to cover major factors that can affect measurements of particles in the real environment, but are not intended to cover all of them. Additional factors that are not considered in this annex include the difference in optical properties between test particles and particles in the real environment, and the uncertainty associated with the determination of theoretical response functions.

NOTE 2 Input quantity is a quantity whose value is used to determine the result of measurement, or a quantity that can otherwise affect a measurement result.

NOTE 3 Although the quantities, Y and X_i, and their estimates, y and x_i, are represented by different symbols in ISO/IEC Guide 98-3, the same symbols are used here, as far as there is no risk of confusion.

NOTE 4 If the estimate of a quantity x_i is obtained from $x_i = \overline{q}$, where $\overline{q}$ is the mean of a series of observations, q_k(k = 1, 2, ..., n), then the standard uncertainty of x_i is evaluated as

$$u(x_i) = \frac{s}{\sqrt{n}} \tag{B.4}$$

Here s is the experimental standard deviation of q_k given by $s=\sqrt{\sum_{k=1}^{n}\left(q_k-\bar{q}\right)^2 \Big/ (n-1)}$, or some other estimate of the standard deviation based on an experiment conducted separately from the measurement of x_i. Uncertainty evaluation based on such statistical analysis of series of observations is called type A evaluation of uncertainty.

NOTE 5 Method of evaluation of uncertainty by means other than the statistical analysis of series of observations is called type B evaluation. Type B evaluation is conducted on the basis of available information such as data given in calibration certificates, instrument specifications, handbooks, and data obtained in the past.

NOTE 6 Formula (B.2) applies to cases where there are no correlations between input quantities. When the correlations are not negligible, extra terms containing correlation coefficients are added to the right-hand side of Formula (B.2). In this document, only cases where correlations are negligible are considered.

NOTE 7 When the mathematical model [Formula (B.1)] takes the form

$$y=C\cdot x_1^{p_1}\cdot x_2^{p_1}\cdots x_N^{p_N} \tag{B.5}$$

where C, p_1, p_2, ... are constants, Formula (B.3) is equivalent to Formula (B6) called the law of propagation of relative uncertainty,

$$\frac{u_c(y)}{|y|}=\sqrt{\sum_{i=1}^{N}\left(p_i\frac{u(x_i)}{x_i}\right)^2} \tag{B.6}$$

NOTE 8 If the probability distribution characterized by y and $u_c(y)$ is a normal distribution, then the interval $y \pm U$ with $k = 2$ encompasses approximately 95 % of the distribution. The coverage factor k can have a different value depending on the actual degrees of freedom, see ISO/IEC Guide 98-3.

B.2 Uncertainty of counting efficiency, η

The standard uncertainty $u(ς)$ of the counting efficiency η obtained according to the procedure described in 7.2 is evaluated based on Formula (B.7) as (see Note 1):

$$u(ς)=ς\sqrt{\left[\left(\frac{u(C_L)}{C_L}\right)^2+\left(\frac{u(C_R)}{C_R}\right)^2\right]} \tag{B.7}$$

The standard uncertainties in the right-hand side of Formula (B.7) are evaluated as follows.

(1) $u(C_R)$

The standard uncertainty $u(C_R)$ is the uncertainty of the certified value C_R of the CRM for particle number concentration. It is obtained from the calibration certificate of the CRM.

(2) $u(C_L)$

Let t_0 denote the sampling time of the LELPC adopted at the counting efficiency test. Conduct measurement of the number concentration of the CRM n times by the LELPC with the same sampling time t_0. The number of repetition n should be larger than five. Let C_{Li}(i = 1, 2, ..., n) denote the measured particle concentration obtained in these repeated measurements. The experimental standard deviation, s_c, of the measured values C_{Li} is calculated by

$$s_C=\sqrt{\frac{\sum_{i=1}^{n}\left(C_{Li}-\bar{C}_L\right)^2}{n-1}} \tag{B.8}$$

