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Capability of detection —

Part 5:
Methodology in the linear and non-linear calibration cases

Capacité de détection —

Partie 5: Méthodologie des étalonnages linéaire et non linéaire

Reference number
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Foreword

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ISO 11843-5 was prepared by Technical Committee ISO/TC 69, *Application of statistical methods*, Subcommittee SC 6, *Measurement methods and results*.

ISO 11843 consists of the following parts, under the general title *Capability of detection*:

— *Part 1: Terms and definitions*

— *Part 2: Methodology in the linear calibration case*

— *Part 3: Methodology for determination of the critical value for the response variable when no calibration data are used*

— *Part 4: Methodology for comparing the minimum detectable value with a given value*

— *Part 5: Methodology in the linear and non-linear calibration cases*

Introduction

Both linear and non-linear calibration functions are encountered in practice. This part of ISO 11843 treats both cases equally in the context of the capability of detection, by paying attention to the probability distributions of the net state variable (measurand), rather than the calibration functions themselves.

The basic concepts of ISO 11843-2 including the probability requirements, α and β, and the linear calibration cases are retained by this part of ISO 11843. In the interval of values between the basic state and minimum detectable value, a linear calibration function may be applied. In this manner, compatibility with ISO 11843-2 is assured.

In the case that an analytical method characterized with a linear calibration function is compared with a method with a non-linear calibration function, this part of ISO 11843 is recommended. In a linear calibration case, ISO 11843-2 and this part of ISO 11843 are both available. ISO 11843-2 which uses the precision profile for the response variable alone will give the same result as this part of ISO 11843 which requires the precision profiles for both the response variable and net state variable, since the precision profile for the response variable is the same as that for the net state variable in the linear case.

Capability of detection —

Part 5: Methodology in the linear and non-linear calibration cases

1 Scope

This part of ISO 11843 is concerned with calibration functions that are either linear or non-linear.

It specifies basic methods to

— construct a precision profile for the response variable, namely a description of the standard deviation (SD) or coefficient of variation (CV) of the response variable as a function of the net state variable,

— transform this precision profile into a precision profile for the net state variable in conjunction with the calibration function, and

— use the latter precision profile to estimate the critical value and minimum detectable value of the net state variable.

The methods described in this part of ISO 11843 are useful for checking the detection of a certain substance by various types of measurement equipment to which ISO 11843-2 cannot be applied. Included are assays of persistent organic pollutants (POPs) in the environment, such as dioxins, pesticides and hormone-like chemicals, by competitive ELISA (enzyme-linked immunosorbent assay), and tests of bacterial endotoxins that induce hyperthermia in humans.

The definition and applicability of the critical value and minimum detectable value of the net state variable are described in ISO 11843-1 and ISO 11843-2. This part of ISO 11843 extends the concepts in ISO 11843-2 to the cases of non-linear calibration.

The critical value, x_c, and minimum detectable value, x_d, are both given in the units of the net state variable. If x_c and x_d are defined based on the distribution for the response variable, the definition should include the calibration function to transform the response variable to the net state variable. This part of ISO 11843 defines x_c and x_d based on the distribution for the net state variable independently of the form of the calibration function. Consequently, the definition is available irrespective of the form of this function, whether it is linear or non-linear.

The calibration function should be continuous, differentiable, and monotonically increasing or decreasing.

A further method is described for the cases where the SD or CV is known only in the neighbourhood of the minimum detectable value.

Examples are provided.

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 3534-1, *Statistics — Vocabulary and symbols — Part 1: General statistical terms and terms used in probability*

ISO 3534-2, *Statistics — Vocabulary and symbols — Part 2: Applied statistics*

ISO 3534-3, *Statistics — Vocabulary and symbols — Part 3: Design of experiments*

ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 11843-1:1997, *Capability of detection — Part 1: Terms and definitions*

ISO 11843-2:2000, *Capability of detection — Part 2: Methodology in the linear calibration case*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 3534 (all parts), ISO 5725-1, ISO 11843-1, ISO 11843-2 and the following apply.

3.1
critical value of the net state variable
x_{c}
value of the net state variable, X, the exceeding of which leads, for a given error probability, α, to the decision that the observed system is not in its basic state

[ISO 11843-1:1997, definition 10]

See Figure 1.

3.2
minimum detectable value of the net state variable
x_{d}
value of the net state variable in the actual state that will lead, with probability $1 - \beta$, to the conclusion that the system is not in the basic state

NOTE Adapted from ISO 11843-1:1997, definition 11 and ISO 11843-1:1997/Cor.1:2003.

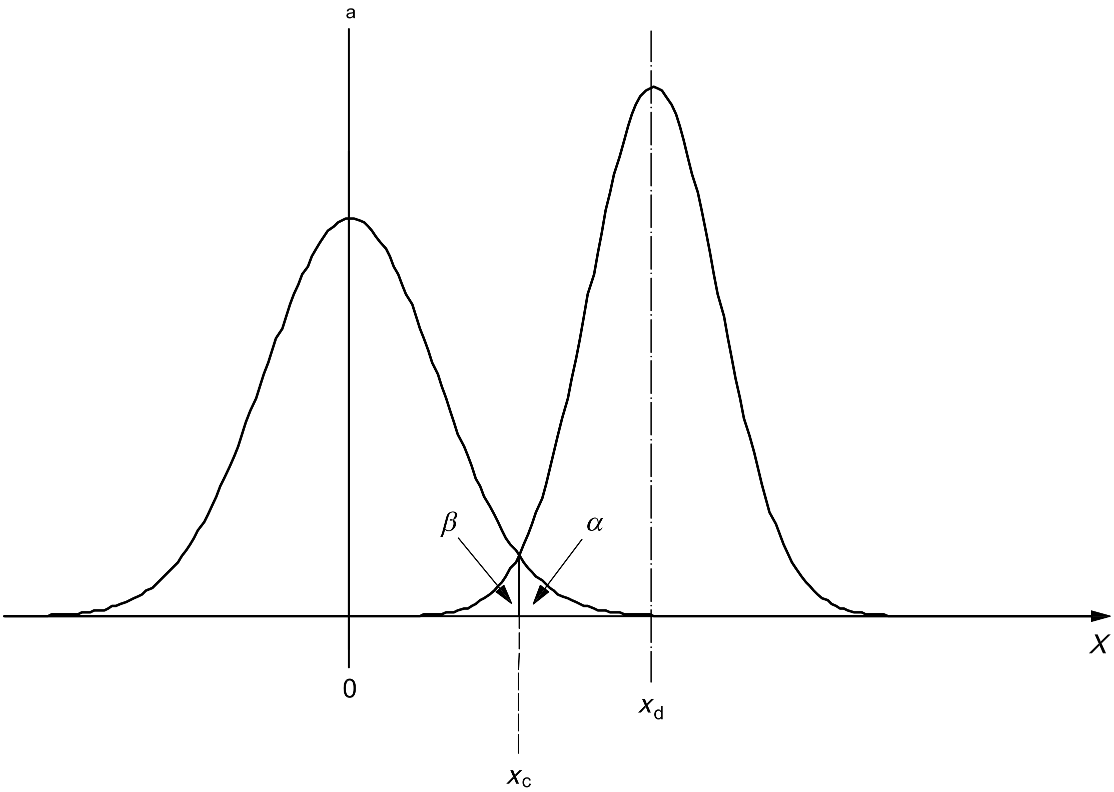
See Figure 1.

3.3
precision
⟨detection capability⟩ standard deviation (SD) of the observed response variable or SD of the net state variable when estimated by the calibration function

NOTE 1 Coefficient of variation (CV) may be used as precision instead of SD where appropriate.

NOTE 2 In this part of ISO 11843, precision is defined under repeatability conditions (ISO 3534-2).

NOTE 3 The terms, precision and precision profile, are used in this part of ISO 11843, rather than imprecision and imprecision profile, because of a tradition to use the former terms in a number of situations.



Key

x_c critical value of the net state variable

x_d minimum detectable value of the net state variable

X net state variable

α probability of an error of the first kind at $X = 0$

β probability of an error of the second kind at $X = x_d$

a Probability density.

NOTE Figure 1 in ISO 11843-1:1997 illustrates the distributions of response variables and the non-linear calibration line. Figure 1 of this part of ISO 11843 includes the distributions of net state variables which are transformed through the slope of the calibration line from the distributions of the response variable shown in ISO 11843-1.

Figure 1 — Distributions of the estimated net state variable in the basic state, $X = 0$, (left) and in the state of x_d (right)

3.4
precision profile
⟨detection capability⟩ mathematical description of the standard deviation or coefficient of variation of the response variable or net state variable as a function of the net state variable

3.5
response variable
Y
variable representing the outcome of an experiment

[ISO 3534-3:1999, definition 1.2]

NOTE 1 For the purposes of ISO 11843, this general definition is understood in the following specialized form: directly observable surrogate for the state variable, Z.

NOTE 2 The response variable, Y, is a random variable in any stage of analysis and if transformed by the calibration function, its precision profile is expressed as the standard deviation and coefficient of variation, $\sigma_X(X)$ and $\rho_X(X)$, respectively, of the net state variable.

3.6
precision profile of response variable
continuous plot in this part of ISO 11843 on the basis of the uncertainty of the response variable which comes from the random properties of analytical steps such as pipetting and instrumental baseline noise, and not from the systematic error often known as the knowledge of instrumental imperfections

3.7
net state variable
X
difference between the state variable, Z, and its value in the basic state, z_0

[ISO 11843-1:1997, definition 4]

NOTE The net state variable, X, is a deterministic variable in the stage where a calibration line is prepared, and the precision profile, expressed as $\sigma_X(X)$ and $\rho_X(X)$, originates from the randomness of the response variable.

4 Precision profile of the net state variable

For experimental or theoretical reasons, the precision (SD or CV) relates to the response variable, Y (rather than the net state variable, X). Therefore, any relevant value of Y needs to be transformed to the corresponding value of X, and the precision transformed accordingly, as shown in Figure 2 [1, 2].

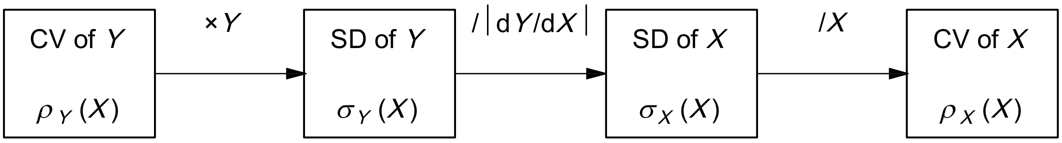


Figure 2 — Transformation of uncertainty from response variable to net state variable

In Figure 3, the SD, $\sigma_Y(X)$, of the response variable can be transformed to the SD, $\sigma_X(X)$, of the net state variable by means of the absolute value of the derivative, $|\mathrm{d}Y/\mathrm{d}X|$, of the calibration function: $\sigma_X(X) = \sigma_Y(X)/|\mathrm{d}Y/\mathrm{d}X|$. The transformation to the CV of X, $\rho_X(X)$, can be formulated as:

$$\rho_X(X) = \frac{\rho_Y(X)Y}{X\left|\dfrac{\mathrm{d}Y}{\mathrm{d}X}\right|} \tag{1}$$

Given $\rho_Y(X)$ as a function of X, the desired quantity, $\rho_X(X)$, can also be written as a function of X with the aid of Equation (1). The use of the absolute value, $|\mathrm{d}Y/\mathrm{d}X|$, extends the application of this part of ISO 11843 to calibration functions that are monotonically decreasing.

NOTE 1 If the calibration function is a straight line passing through the origin ($Y = aX$), the precision profile, $\rho_X(X)$, of the net state variable is equal to the precision profile, $\rho_Y(X)$, of the response variable. Note that $Y/X = |\mathrm{d}Y/\mathrm{d}X| = a$, as $Y = aX$.

NOTE 2 Equation (1) is not valid for $X = 0$, but covers most practical situations where the coefficient of variation, $\rho_X(X)$, diverges to infinity with decreasing X as long as the SD, $\sigma_X(X)$ (= $\rho_Y(X)Y/|\mathrm{d}Y/\mathrm{d}X|$), of the net state variable is finite.

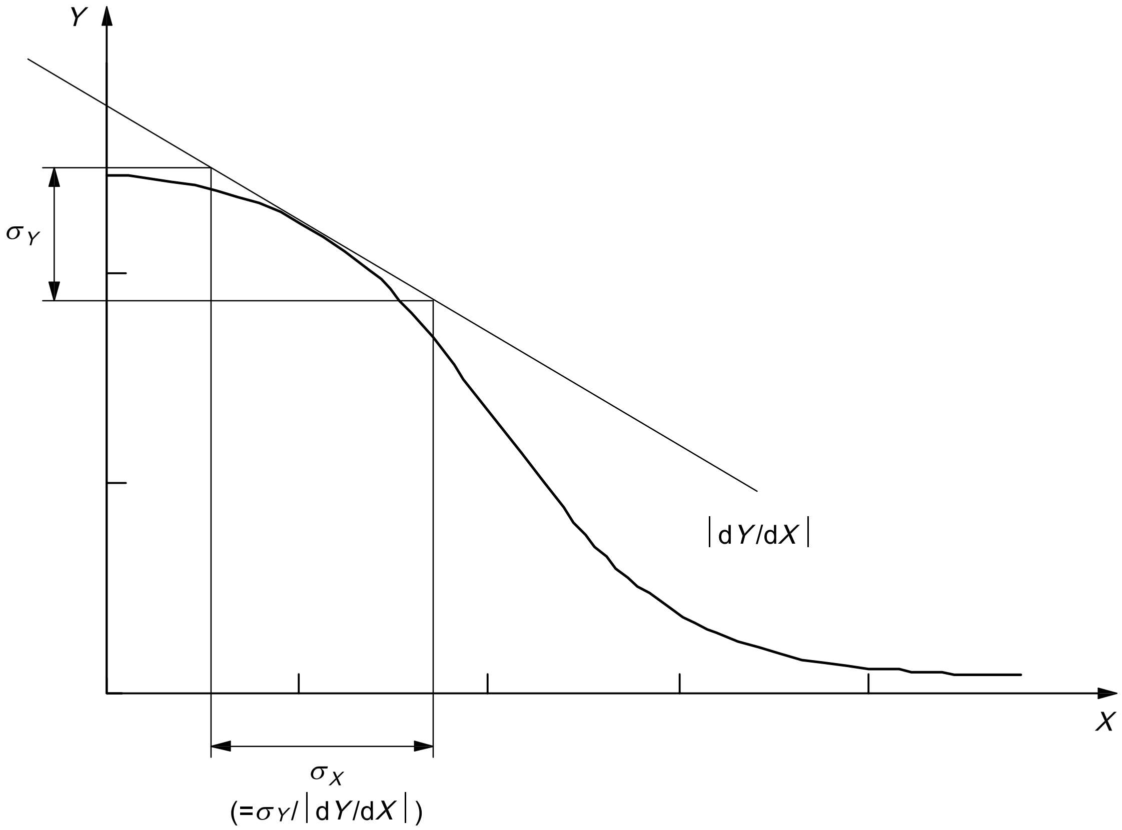


Figure 3 — Transformation from the SD, σ_Y, of the response variable to the SD, σ_X, of the net state variable by means of the absolute value of the derivative, $|dY/dX|$, of the calibration curve

5 Critical value and minimum detectable value of the net state variable

5.1 General

All definitions below are based on a probability distribution for the net state variable.

The critical value, x_c, is defined as:

$$x_c = k_c \sigma_X(0) \quad (2)$$

where

k_c denotes a coefficient to specify α;

$\sigma_X(0)$ is the SD at $X = 0$.

If the relationship that $\sigma_X(0) = \sigma_Y(0)/|dY/dX|$ is used, Equation (2) can be described as $x_c = k_c\sigma_Y(0)/|dY/dX|$.

The minimum detectable value, x_d, is defined as

$$x_d = x_c + k_d \sigma_X(x_d) \quad (3)$$

where

k_d denotes a coefficient to specify β;

$\sigma_X(x_d)$ is the SD at $X = x_d$ (see Figure 1).

The precision profile, $\sigma_X(X)$, (see 3.4) is necessary to determine both the critical value, x_c, and minimum detectable value, x_d.

NOTE 1 If the net state variable is normally distributed, coefficients, $k_c = k_d = 1{,}65$, specify the probabilities, $\alpha = \beta = 5$ %.

NOTE 2 Under the special assumption that $\sigma_X(X)$ is constant ($\sigma_X(X) = \sigma_X$) and $k_c = k_d = 1{,}65$, Equations (2) and (3) can simply be written as $x_c = 1{,}65\sigma_X$ and $x_d = 3{,}30\sigma_X$.

5.2 Calculation relating to probability α

If the SD is for $X = 0$, then $\sigma_X(0)$, is used instead of $\sigma_X(x_d)$, the definitions of x_c and x_d take the forms:

$$x_c = k_c\sigma_X(0) \tag{4}$$

$$x_d = (k_c + k_d)\sigma_X(0) \tag{5}$$

In this case, Equation (4) is the same as Equation (2) and the probability, α, is equal to the general definition. However, the probability, β, can be different from the original β. The full precision profile, $\sigma_X(X)$, is not required for this calculation.

NOTE Under the special assumption that $\sigma_X(X)$ is constant $[\sigma_X(X) = \sigma_X]$ and $k_c = k_d = 1{,}65$, Equations (4) and (5) can simply be written as $x_c = 1{,}65\sigma_X$ and $x_d = 3{,}30\sigma_X$.

5.3 Calculation relating to probability β

When $\sigma_X(x_d)$ is used instead of $\sigma_X(0)$ in 5.2, the definitions of x_c and x_d take the forms:

$$x_c = k_c\sigma_X(x_d) \tag{6}$$

$$x_d = (k_c + k_d)\sigma_X(x_d) \tag{7}$$

In this case, the probability, β, is equal to the general definition, but the probability, α, can be different from the original, α.

NOTE Under the special assumption that $\sigma_X(X)$ is constant $[\sigma_X(X) = \sigma_X]$ and $k_c = k_d = 1{,}65$, Equations (6) and (7) can simply be written as $x_c = 1{,}65\sigma_X$ and $x_d = 3{,}30\sigma_X$.

5.4 Differential method

The definition of 5.3 has a practical advantage, if expressed as Equation (10). Equation (7) can be written as:

$$\rho_X(x_d) = \sigma_X(x_d)/x_d = 1/(k_c + k_d) \tag{8}$$

This equation gives the CV of the net state variable at $X = x_d$. An advantage of Equation (8) is that the minimum detectable value, x_d, can be determined as the value of the net state variable at which the CV of the expected net state variable is $1/(k_c + k_d) \times 100$ %. The continuous precision profile, $\sigma_X(X)$, is necessary for x_c and x_d.

While the slope, dY/dlg X, of the semi-logarithmic plot (Y versus lg X) of a calibration function varies depending on the net state variable, X, the slope takes a specific value at the minimum detectable value:

$$\left|\frac{\mathrm{d}Y}{\mathrm{d}\lg X}\right|_{X=x_\mathrm{d}} = 2{,}303\left(k_\mathrm{c}+k_\mathrm{d}\right)\times\sigma_Y\left(x_\mathrm{d}\right) \qquad (9)$$

where the left side member denotes the absolute value of the derivative, |dY/dlg X|, at $X = x_\mathrm{d}$ (ln 10 = 2,303). This equation is a general rule for calibration curves and holds good irrespective of the shape of the calibration curve (linear or non-linear). The derivation of Equation (9) is given in Annex B.

NOTE 1 If $k_\mathrm{c} = k_\mathrm{d} = 1{,}65$, Equation (8) can be written as $\sigma_X(X) = 1/3{,}30 = 30$ %. x_d is located at X, the CV of which is 30 %.

NOTE 2 If $k_\mathrm{c} = k_\mathrm{d} = 1{,}65$, Equation (9) can be written as

$$\left|\frac{\mathrm{d}Y}{\mathrm{d}\lg X}\right|_{X=x_\mathrm{d}} = \frac{\sigma_Y\left(x_\mathrm{d}\right)}{0{,}132} \qquad (10)$$

where the constant 0,132 is determined as 1/(3,3 × 2,303).

6 Examples

6.1 General

Subclauses 6.2 and 6.3 focus on how to estimate the precision profile (see 3.4) which is expressed in terms of the SD or CV of the response variable. The final quantity, $\rho_X(X)$, can be transformed from the continuous plot of the SD or CV of the response variable as shown in Clause 4.

The example in 6.4 shows an application of the differential method to competitive ELISA. In 6.4, it is demonstrated that the calibration function of competitive ELISA is usually non-linear, but the linearity assumption is valid at levels close to the minimum detectable value.

6.2 Law of propagation of uncertainty

A competitive ELISA for 17α-hydroxyprogesterone is taken as an example. The experimental procedures of this system are shown in Figure 4. This assay is carried out on a microplate which has 96 wells. A calibration line is made for the microplate and the actual analysis of samples is performed in the other wells of the same microplate. Here, the within-plate uncertainty is examined.

The uncertainty of the competitive ELISA basically comes from the competitive reaction between the sample and labeled antigen. The response variable, Y (here, absorbance measurement), is proportional to the labeled antigen combined with the antibody (antiserum) on the surface of a well in the microplate: [1]

$$Y \propto \frac{G}{X+G}B$$

where

X denotes the amount of sample (net state variable);

G is the amount of labelled antigen;

B is the amount of antibody.

Based on the application of the law of propagation of uncertainty [3] to the procedures of the assay, the squared CV, $\rho_Y(X)^2$, of the response variable, Y, is derived: [1]

$$\rho_Y(X)^2 = \frac{X^2}{(X+G)^2} + \left(r_G^2 + r_X^2\right) + r_B^2 + r_S^2 + \left(\frac{\sigma_W}{Y}\right)^2 \qquad (11)$$

where

X is the amount of sample (net state variable);

Y is absorbance measurement (response variable) and can be replaced by a calibration function;

G is the amount of labeled antigen (0,1 µg/l);

r_X is the CV of pipetted volumes of sample (0,9 %);

r_G is the CV of pipetted volumes of labeled antigen (0,9 %);

r_B is the CV of pipetted volumes of antiserum (1,9 %);

r_S is (2/3) × (CV of pipetted volumes of chromogen-substrate solution) where the coefficient 2/3 is used to transform the volume error of the pipette to the essential error of chromogen production which occurs on the surface of the well in a microplate (0,6 %);

σ_W is the SD of the absorbance measurements among the wells of a microplate and is constant as long as the within-plate uncertainty is concerned (0,002 absorbance).

The final quantity of precision, $\rho_X(X)$, can be calculated from Equation (11) as shown in Figure 2.

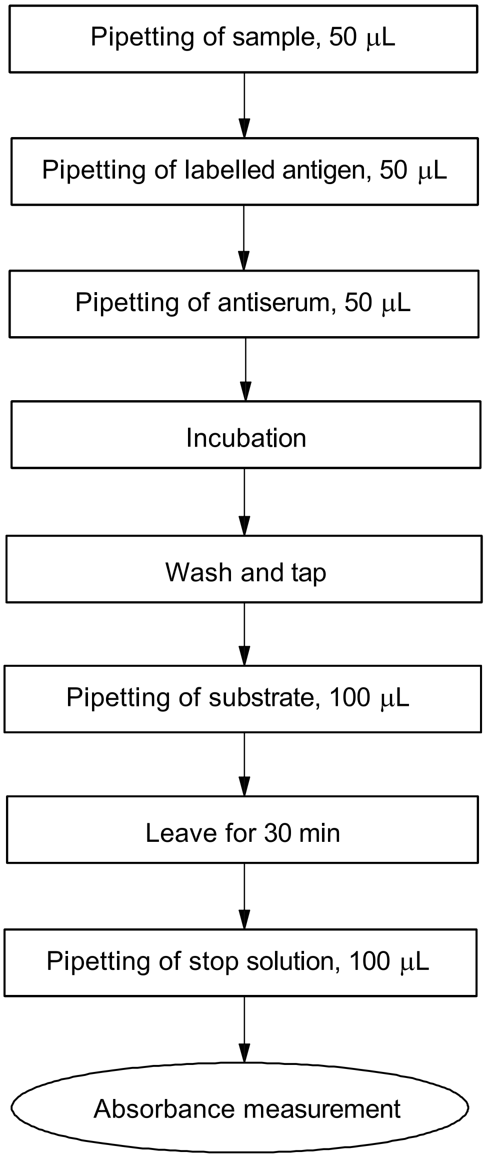
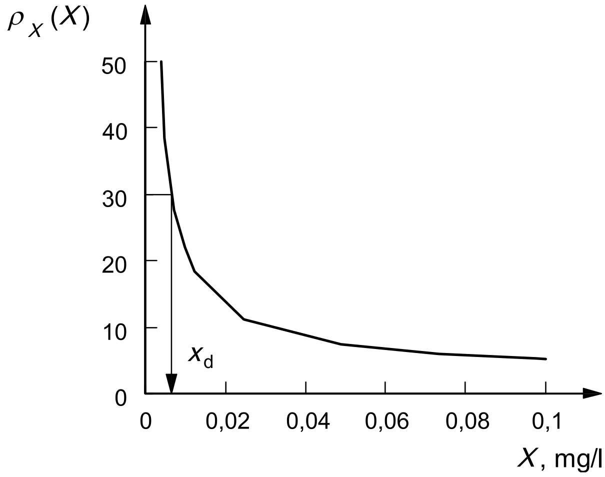


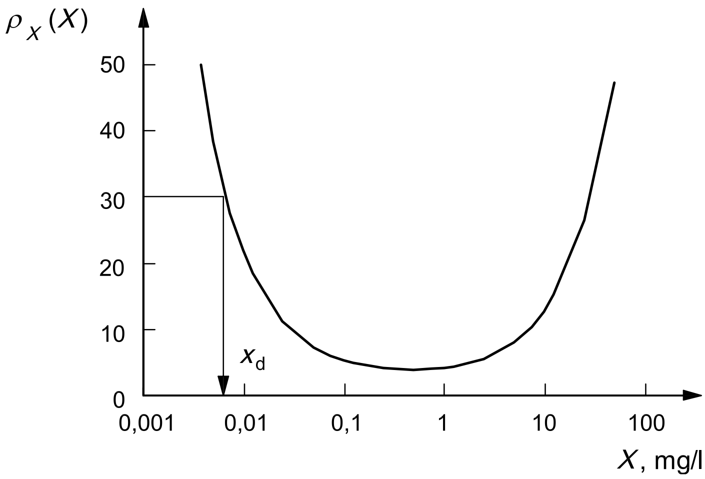
Figure 4 — Experimental procedures of a competitive ELISA

The precision profile, $\rho_X(X)$, of this example is given in Figure 5. The CV, $\rho_X(X)$, is calculated from Equation (11) with the actual parameters described above and is expressed as a percentage. If the definition of 5.3 is adopted, the minimum detectable value, x_d, can be determined on the precision profile (see the arrow of Figure 5). The meaning of 30 % CV is given in Note 1 of 5.4.

The precision profiles in the normal scale and semi-logarithmic scale give the same minimum detectable value. Figure 5 b) excludes the point for $X = 0$ and also the CV therein. However, this poses no problem, theoretical or practical, since the requirement of this part of ISO 11843 for the minimum detectable value is a CV value expressed as the precision profile around the minimum detectable value.



a) Normal scale



b) Semi-logarithmic plot

Figure 5 — CV of net state variable, $\rho_X(X)$, (precision profile) and minimum detectable value, x_d, in the normal scale and semi-logarithmic plot for a competitive ELISA for 17α-hydroxyprogesterone

6.3 Model fitting

In immunoassays, the variances of the response variable can be approximated by the exponential model: [2]

$$\sigma_Y(X)^2 \propto Y^j \tag{12}$$

where $\sigma_Y(X)$ denotes the SD of the response variable, Y. If $j = 0$, the variance is constant. If $j = 1$, the variance is proportional to the response variable. If $j = 2$, the CV, $\rho_Y(X)$, of the response variable is constant. The proportionality constant can be determined by least squares fitting.

6.4 Application to competitive ELISA

In competitive ELISA, the standardized calibration curve, referred to as B/B_0, is often used and Equation (10) can be written as: [4]

$$\left| \mathrm{d}\left(\frac{1}{1+\left(\frac{X}{C_2}\right)^{C_1}} \right) \Big/ \mathrm{d}\lg X \right|_{X=x_\mathrm{d}} = \frac{\rho_Y(x_\mathrm{d})}{0{,}132} \qquad (13)$$

where $\rho_Y(x_\mathrm{d})$ denotes the response CV at x_d. The derivation is given in Annex C.

The minimum detectable value of the net state variable can be found from the slope specified by Equation (13). Figure 6 shows the semi-logarithmic B/B_0 curve in a competitive ELISA for 17α-hydroxyprogesterone (the same as Example 6.2). If the response CV which was observed to be 1,9 % CV at a low sample concentration is used for the approximation [$\approx \rho_Y(x_\mathrm{d})$], Equation (13) gives 0,15 (= 0,019 / 0,132).

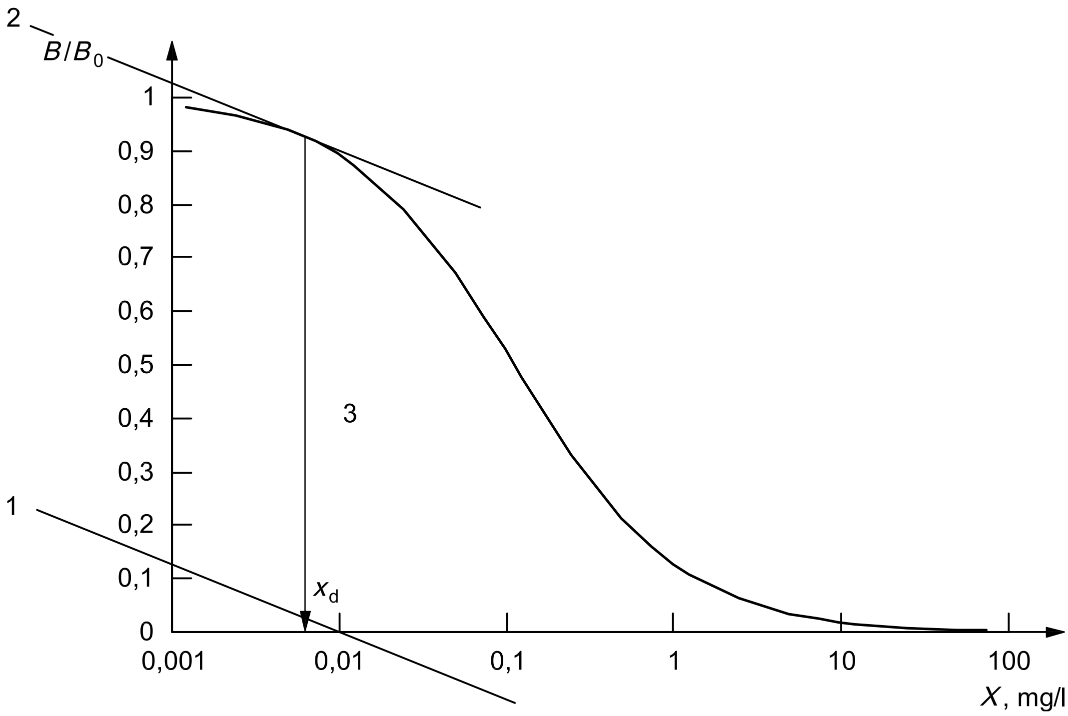
The graphical estimation of x_d is written as follows (see Figure 6):

— Step 1: draw a straight line having the slope calculated by Equation (13) with the aid of the scales in the bottom left-hand corner;

— Step 2: draw the tangent which touches the B/B_0 curve with the same slope as in Step 1;

— Step 3: drop the perpendicular from the point of contact to the X-axis.

The point of intersection of the perpendicular and X-axis corresponds to the x_d. This method provides almost the same result as that of the example of 6.2 (compare Figures 5 and 6).



Key

1, 2, 3 steps 1, 2 and 3 as described in 6.4

Figure 6 — Semi-logarithmic plot of B/B_0 curve in a competitive ELISA for 17α-hydroxyprogesterone

Annex A
(normative)

Symbols and abbreviations used in this part of ISO 11843

SD	standard deviation
CV	coefficient of variation (SD divided by the mean)
POP	persistent organic pollutant
ELISA	enzyme-linked immunosorbent assay
X	net state variable
Y	response variable
x_c	critical value of net state variable
x_d	minimum detectable value of net state variable
k_c	coefficient to specify α
k_d	coefficient to specify β
α	probability of an error of the first kind at $X = 0$
β	probability of an error of the second kind at $X = x_\mathrm{d}$
$\sigma_Y(X)$	SD of response variable as a function of X
$\rho_Y(X)$	CV of response variable as a function of X
$\sigma_X(X)$	SD of net state variable as a function of X
$\rho_X(X)$	CV of net state variable as a function of X
$\lvert \mathrm{d}Y/\mathrm{d}X \rvert$	derivative of calibration function
B/B_0	ratio of measurements for arbitrary dose to measurements for zero dose

Annex B
(informative)

Derivation of Equation (9)

The transformation equation [Equation (1)] can be used to change the equation of the x_d definition [Equation (7)] as shown below:

$$\sigma_X(X) = \frac{\sigma_Y(X)}{\left|\frac{dY}{dX}\right|}$$

$$x_d = (k_c + k_d) \times \frac{\sigma_Y(x_d)}{\left|\frac{dY}{dX}\right|_{X=x_d}} = (k_c + k_d) \times \frac{\sigma_Y(x_d)}{\left|\frac{dY}{d\ln X}\right|_{X=x_d} \frac{1}{x_d}} = (k_c + k_d) \times \frac{\sigma_Y(x_d) x_d}{\left|\frac{dY}{d\ln X}\right|_{X=x_d}}$$

where the absolute value of the derivative is used in the case the slope is negative. The unknown variables, x_d, can be eliminated from the above equation:

$$\left|\frac{dY}{d\ln X}\right|_{X=x_d} = (k_c + k_d) \times \sigma_Y(x_d)$$

The conversion of the natural logarithm into the common logarithm for practical purposes ($\ln X = 2{,}303 \lg X$) can lead to the objective equation [Equation (9)]. Also see Reference [4].

Annex C
(informative)

Derivation of Equation (13)

In competitive ELISA, the calibration curve is usually expressed as the four-parameter logistic function:

$$Y = \frac{C_0 - C_3}{1 + \left(\frac{X}{C_2}\right)^{C_1}} + C_3$$

and its standard form is known as B/B_0:

$$\frac{B}{B_0} = \frac{1}{1 + \left(\frac{X}{C_2}\right)^{C_1}}$$

where C_0, C_1, C_2 and C_3 are coefficients to be determined by least squares fitting to real calibration data. The substitution of the relationship $\mathrm{d}Y = (C_0 - C_3)\mathrm{d}B/B_0$ into Equation (10) yields:

$$\left| \frac{\mathrm{d}B/B_0}{\mathrm{d}\lg X} \right| = \frac{\sigma_Y(X)/(C_0 - C_3)}{0{,}132}$$

Since coefficient C_0 denotes the largest response for the blank sample ($X = 0$) and C_3 the smallest one at infinite concentration ($X = \infty$), $\sigma_Y(X)/(C_0 - C_3)$ is approximately equal to $\sigma_Y(X)/C_0$. Let $\rho_Y(X)$ be defined as:

$$\rho_Y(X) = \frac{\sigma_Y(X)}{C_0 - C_3} \approx \frac{\sigma_Y(X)}{C_0}$$

where $\sigma_Y(X)/C_0$ means the CV of blank responses, $\rho_Y(0)$. The last two equations lead to Equation (13). Also see Reference [4].

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