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Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods

AMENDMENT 1

Microbiologie des aliments — Protocole pour la validation des méthodes alternatives

AMENDEMENT 1

Reference number
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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.

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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Amendment 1 to ISO 16140:2003 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods

AMENDMENT 1

Page 22, 6.3

Replace the existing text with that commencing on p. 2.

Page 61, Annex Q

Replace the existing text with that commencing on p. 9.

Page 74, Bibliography

Insert Annexes V and W commencing on p. 11 before the bibliography.

Additions to the bibliography are given on p. 18.

6.3 Interlaboratory study

6.3.1 General

An interlaboratory study aims to **determine the comparative performance characteristics** (trueness and precision characteristics) of the alternative method against the reference method.

Guidelines and requirements for organizing, dispatching and conducting the interlaboratory study are given in Annex H and in ISO 5725-2.

At least eight laboratories shall participate in an interlaboratory study.

The organizing laboratory is responsible for the preparation of the test protocol and a data sheet (see below) to be used by each laboratory for recording all measurement results and critical experimental conditions (see H.3).

The analyst in each collaborating laboratory shall demonstrate competence in the use of the alternative method and of the reference method prior to participating in the study.

In microbiology, the data $\{y\}$ of repeated measurements do not always show a normal (Gaussian) distribution. Therefore, the distribution of these data should be checked for normality if more than 30 values are available at the same level. In order to get a more symmetric distribution, take logarithms of the counts.

The data obtained from interlaboratory studies often contain outliers, i.e. measurement values that deviate so much from comparable measurements that they are considered inconsistent. If they are retained in the data set, the trueness and precision characteristics (averages, standard deviations, etc.) obtained with classical methods of statistical analysis are unreliable. Therefore, ISO 5725-2 includes **outlier tests** (Cochran, Grubbs) in order to detect and eventually discard outliers and to obtain reliable trueness and precision characteristics. Often this causes disputes as to which of the outliers should be discarded from or retained in the statistical analysis. In order to avoid such disputes, **robust estimates** of the trueness and precision characteristics are used in this International Standard. Since they are insensitive to any extreme values, they always use the complete data set obtained from the interlaboratory study.

However, each extreme value should be checked for a clerical error in transcribing the measurement result, an error in computation, a slip in performing the measurement or an analysis of the wrong sample. If possible, such values should be replaced by the correct values. **Other extreme measurement results or laboratories reporting extreme values are not excluded from the statistical analysis unless exclusion is based on sound microbiological reasons.**

6.3.2 Terms and definitions

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

6.3.2.1
accuracy
closeness of agreement between a test result or measurement result and the true value

NOTE 1 In practice, the accepted reference value is substituted for the true value.

[ISO 3534-2:2006[13], 3.3.1]

NOTE 2 Accuracy refers to a combination of trueness and precision.

6.3.2.2
trueness
closeness of agreement between the expectation of a test result or a measurement result and the true value

NOTE 1 The measure of trueness is usually expressed in terms of bias.

NOTE 2 In practice, the accepted reference value is substituted for the true value.

[ISO 3534-2:2006[13], 3.3.3]

6.3.2.3
precision
closeness of agreement between independent test/measurement results obtained under stipulated conditions

NOTE Quantitative measures of precision depend critically on the stipulated conditions. Repeatability conditions and reproducibility conditions are particular sets of extreme stipulated conditions.

[ISO 3534-2:2006[13], 3.3.4]

6.3.2.4
repeatability
precision under repeatability conditions

[ISO 3534-2:2006[13], 3.3.5]

6.3.2.5
repeatability conditions
observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in the same test or measuring facility by the same operator using the same equipment within short intervals of time

[ISO 3534-2:2006[13], 3.3.6]

6.3.2.6
repeatability standard deviation
standard deviation of test result or measurement results obtained under repeatability conditions

[ISO 3534-2:2006[13], 3.3.7]

6.3.2.7
repeatability limit
r
value less than or equal to which the absolute difference between two measurement results obtained under repeatability conditions is expected to be with a probability of 95 %

NOTE Adapted from ISO 3534-2:2006[13], 3.3.9.

6.3.2.8
reproducibility
precision under reproducibility conditions

[ISO 3534-2:2006[13], 3.3.10]

6.3.2.9
reproducibility conditions
observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in different test or measuring facilities with different operators using different equipment

[ISO 3534-2:2006[13], 3.3.11]

6.3.2.10
reproducibility standard deviation
standard deviation of test results or measurement results obtained under reproducibility conditions

[ISO 3534-2:2006[13], 3.3.12]

6.3.2.11
reproducibility limit
R
value less than or equal to which the absolute difference between two measurement results obtained under reproducibility conditions is expected to be with a probability of 95 %

NOTE Adapted from ISO 3534-2:2006[13], 3.3.14.

6.3.3 Measurement protocol and samples

The protocol is the following.

— One relevant food matrix is used (see Annex B).

— The analyte concentrations should be chosen to cover at least the lower, middle and upper levels of the entire range of the alternative method. The organizing laboratory shall assure that the samples are homogeneous (Reference [14]). A negative control should also be included.

— Artificial contamination of a food sample with the target analyte may be used.

— To compare the alternative method with the reference method the same samples shall be used for each method. Four sub-samples from each level (or two aliquots, each measured by both methods) are prepared for each laboratory. These are blind coded but labelled so that two are measured by the reference method and two are measured by the alternative method.

— Liquid samples (compared to solid samples) give greater assurance of homogeneity if prepared and dispatched without change in microbiological content and used correctly. In specific cases, it could be necessary to subdivide the samples immediately before measurement with both methods.

— The measurements shall be performed in each collaborative laboratory and the organizing laboratory at a stipulated date using common batches of media and kits.

— The rounding of results shall be set by the organizing laboratory.

— For each level j, the results of the interlaboratory study shall be presented as shown in Table 9.

Table 9 — Presentation of the results of the interlaboratory study at level j

Laboratory i	Reference method (coded)		Alternative method (coded)	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1				
2				
…				
p				

6.3.4 Determination of the trueness and precision characteristics

6.3.4.1 General

For each level j, the following trueness and precision characteristics are determined:

— the median of the laboratory means of the measurement results of the reference method and of the alternative method (for the estimation of bias);

— the repeatability standard deviation of the reference method and of the alternative method based on the calculation of Rousseeuw's Q_n (see Annex Q);

— the reproducibility standard deviation of the reference method and of the alternative method based on Rousseeuw's Q_n (see Annex Q).

6.3.4.2 Calculations

For each level j, perform the calculations according to the following steps. In the following text, the subscript j is omitted.

Step 1. With the measurement results for the reference method (Table 9, columns 2 and 3), prepare Table 10 for each level j.

Table 10 — Measurement results, means and deviations at level j (for the reference method or for the alternative method)

1	2	3	4	5	6
Laboratory	Measurement		Mean	Deviation from the mean	
1	y_{11}	y_{12}	$\bar{y}_1$	d_{11}	d_{12}
2	y_{21}	y_{22}	$\bar{y}_2$	d_{21}	d_{22}
…	…	…	…	…	…
i	y_{i1}	y_{i2}	$\bar{y}_i$	d_{i1}	d_{i2}
…	…	…	…	…	…
p	y_{p1}	y_{p2}	$\bar{y}_p$	d_{p1}	d_{p2}

In Table 10,

$$\bar{y}_i = \frac{y_{i1} + y_{i2}}{2}$$

is the mean of the two measurements in laboratory i and

$$d_{i1} = y_{i1} - \bar{y}_i = \frac{y_{i1} - y_{i2}}{2}$$

$$d_{i2} = y_{i2} - \bar{y}_i = \frac{y_{i2} - y_{i1}}{2}$$

are the deviations of these two measurements from their mean.

Step 2. Use the deviations of Table 10, columns 5 and 6 as a series of $n = 2p$ values and calculate Rousseeuw's bias corrected scale estimator Q_{intra}:

$$Q_{\text{intra}} = c_n Q_n \text{ with } n = 2p$$

as described in Annex Q.

Step 3. Calculate Rousseeuw's bias corrected scale estimator Q_{inter}:

$$Q_{\text{inter}} = c_n Q_n \text{ with } n = p$$

of the laboratory means of Table 10, column 4 as described in Annex Q.

Step 4. Calculate the median m of the laboratory means of the results of the reference method (Table 10, column 4).

Step 5. Calculate the repeatability standard deviation s_r:

$$s_r = \sqrt{2}\, Q_{\text{intra}}$$

Calculate the coefficient of variation[1)] of repeatability, $C_{V,r}$:

$$C_{V,r} = \frac{s_r}{m}$$

Calculate the repeatability limit r:

$$r = 2{,}8 s_r$$

Step 6. Calculate the between laboratories standard deviation s_{L}:

$$s_{\text{L}} = \sqrt{Q_{\text{inter}}^2 - Q_{\text{intra}}^2}$$

or 0, if $Q_{\text{inter}}^2 - Q_{\text{intra}}^2 \leq 0$.

Calculate the reproducibility standard deviation s_R:

$$s_R = \sqrt{s_{\text{L}}^2 + s_r^2}$$

Calculate the coefficient of variation[1)] of reproducibility, $C_{V,R}$:

$$C_{V,R} = \frac{s_R}{m}$$

Calculate the reproducibility limit, R:

$$R = 2{,}8 s_R$$

1) The predecessor term to “coefficient of variation” used in ISO 16140:2003, “relative standard deviation” is deprecated in ISO 3534-1:2006, 2.38, Note 2. In this amendment, the symbol C_V is used in place of RSD.

Step 7. Repeat steps 1 to 6 for the alternative method.

After having carried out the calculations of steps 1 to 7 for each level j, combine the results of the determinations for all q levels, as in Table 11.

A functional relationship between the repeatability standard deviation or the reproducibility standard deviation and the median level m may exist. Methods for their establishment can be found in ISO 5725-2.

Table 11 — Results of the statistical analysis

Level	Reference method			Alternative method		
	median	repeatability s.d.	reproducibility s.d.	median	repeatability s.d.	reproducibility s.d.
1	$m_{1,\text{ref}}$	$s_{r1,\text{ref}}$	$s_{R1,\text{ref}}$	$m_{1,\text{alt}}$	$s_{r1,\text{alt}}$	$s_{R1,\text{alt}}$
…	…	…	…	…	…	…
j	$m_{j,\text{ref}}$	$s_{rj,\text{ref}}$	$s_{Rj,\text{ref}}$	$m_{j,\text{alt}}$	$s_{rj,\text{alt}}$	$s_{Rj,\text{alt}}$
…	…	…	…	…	…	…
q	$m_{q,\text{ref}}$	$s_{rq,\text{ref}}$	$s_{Rq,\text{ref}}$	$m_{q,\text{alt}}$	$s_{rq,\text{alt}}$	$s_{Rq,\text{alt}}$

6.3.5 Scrutiny of the measurement results for consistency

In order to identify measurement results or laboratories that are inconsistent with the other measurement results or laboratories, two graphical consistency techniques are applied, i.e. Mandel's h- and k- statistics in a robustified version.

6.3.5.1 For the reference method, calculate the between-laboratory consistency statistic, Mandel's h_{ij}, for each of the p laboratories, $i = 1 \ldots p$, and for each of the q levels $j = 1 \ldots q$ by dividing the mean $\bar{y}_{ij}$ (Table 10, column 4, for each level j) minus the median m_j (Table 11, column 2) for that level j by Rousseeuw's bias corrected scale estimator $Q_{\text{inter},j}$ for that level j (as determined in Step 3),

$$h_{ij} = \frac{\bar{y}_{ij} - m_j}{Q_{\text{inter},j}}$$

Plot the $p\,q$ values (h_{ij}) sequentially in the order: laboratory 1, levels 1 … q, laboratory 2, levels, 1 … q, … to laboratory p, levels 1 … q (see Figure W.1).

Add the indicators for Mandel's h at the 5 % and 1 % significance levels (Table V.1) as horizontal lines to this plot. In case of between-laboratory consistency, only 5 % or 1 %, respectively, of the values h_{ij} are expected to lie above these horizontal lines.

6.3.5.2 For the reference method, calculate the within-laboratory consistency statistic, Mandel's k_{ij}, for each of the p laboratories, $i = 1 \ldots p$ and for each of the q levels $j = 1 \ldots q$ by dividing the absolute difference between the two repeated measurements, $|y_{ij1} - y_{ij2}|$ (Table 10, columns 2 and 3) by √2 multiplied by the repeatability standard deviation s_{rj} for that level j,

$$k_{ij} = \frac{|\,y_{ij1} - y_{ij2}\,|}{\sqrt{2}s_{rj}}$$

Plot the $p\,q$ values (k_{ij}) sequentially in the order: laboratory 1, levels 1 … q, laboratory 2, levels, 1 …q, to laboratory p, levels 1 … q (see Figure W.2).

Add the indicators for Mandel's k at the 5 % significance and 1 % significance levels (Table V.1) as horizontal lines to this plot. In case of within-laboratory consistency, only 5 % or 1 %, respectively, of the values k_{ij} are expected to lie above these horizontal lines.

6.3.5.3 Repeat 6.3.5.1 and 6.3.5.2 for the alternative method.

6.3.5.4 Examination of the h and k plots may indicate that specific laboratories exhibit patterns of results that are markedly different from the others in the study. This is indicated by consistently high or low within-laboratory variation and/or extreme laboratory means across levels. If this occurs, the specific laboratory should be contacted to try to ascertain the cause of the discrepant behavior.

6.3.6 Comparison of the trueness and precision characteristics of the reference method and the alternative method

6.3.6.1 Bias of the alternative method

In order to estimate the bias of the alternative method with respect to the reference method at level j, compute the differences of the means $\bar{y}_{ij,\text{alt}}$ of the two measurement values obtained with the alternative method (Table 10, column 4 prepared for the alternative method at level j) and the means $\bar{y}_{ij,\text{ref}}$ of the two measurement values obtained with the reference method (Table 10, column 4 prepared for the reference method at level j),

$$D_{ij} = \bar{y}_{ij,\text{alt}} - \bar{y}_{ij,\text{ref}}$$

Compute the median, $m_i(D_{ij})$ and Rousseeuw's scale estimator $Q_{\text{diff}} = c_p Q_n(D_{ij})$ of these p differences as described in Annex Q.

If the value, at level j, of

$$t = \frac{\left| m_i(D_{ij}) \right|}{\sqrt{\pi/(2p)}\, Q_{\text{diff}}}$$

is larger than 2, the alternative method is significantly biased with respect to the reference method at this level j.

6.3.6.2 Comparison of the repeatability standard deviations

If, at level j, the ratio $s_{rj,\text{alt}}/s_{rj,\text{ref}}$ of the repeatability standard deviations of the alternative method and the reference method is larger than 2, the precision under repeatability conditions of the alternative method is considered to be lower than that of the reference method. If this ratio $s_{rj,\text{alt}}/s_{rj,\text{ref}}$ is smaller than 0,5, the precision under repeatability conditions of the alternative method is considered to be greater than that of the reference method.

6.3.6.3 Comparison of the reproducibility standard deviations

If, at level j, the ratio $s_{Rj,\text{alt}}/s_{Rj,\text{ref}}$ of the reproducibility standard deviations of the alternative method and the reference method is larger than 2, the precision under reproducibility conditions of the alternative method is considered to be lower than that of the reference method. If this ratio $s_{Rj,\text{alt}}/s_{Rj,\text{ref}}$ is smaller than 0,5, the precision under reproducibility conditions of the alternative method is considered to be greater than that of the reference method.

Annex Q
(normative)

Calculation of Rousseeuw's scale estimator Q_n

Given a series of n observed values y_1, y_2 ... y_n, Rousseeuw's scale estimator Q_n is based on the absolute differences of all pairs of observed values,

$$| y_i - y_j | \qquad i = 1, 2 \ldots n-1, \qquad j = 2, 3 \ldots n \qquad i < j$$

or written explicitly,

$$\begin{aligned} | y_1 - y_2 |, | y_1 - y_3 | \ldots | y_1 - y_{n-1} |, | y_1 - y_n |, \\ | y_2 - y_3 | \ldots | y_2 - y_{n-1} |, | y_2 - y_n |, \\ , \ldots\ldots\ldots\ldots\ldots\ldots\ldots, \\ | y_{n-1} - y_n | \end{aligned}$$

These $n(n - 1)/2$ absolute differences are arranged in ascending order and the lth smallest absolute difference of this ordered series is picked as

$$Q_n = (| y_i - y_j |_{i<j})_{(l)}$$

where

$$l = \frac{f(f-1)}{2}$$

and

$$f = \begin{cases} n/2+1 & \text{if } n \text{ is even} \\ (n+1)/2 & \text{if } n \text{ is odd} \end{cases}$$

In order to get an unbiased estimator of the theoretical standard deviation in case of a normal distribution of the observed values, Q_n has to be multiplied by a bias correction factor

$$c_n = \begin{cases} 2,2219 \dfrac{n}{n+1,4} & \text{if } n \text{ is odd} \\ 2,2219 \dfrac{n}{n+3,8} & \text{if } n \text{ is even} \end{cases}$$

EXAMPLE Given a series of $n = 5$ observed values, 34, 41, 67, 53, 42, these values can be written in a scheme, consisting of a row and a column for each observed value. In the scheme, the absolute deviations of the sample values against each other can be entered.

	34	41	67	53	42
34		7	33	19	8
41			26	12	1
67				14	25
53					11
42					

1, 7, 8, 11, 12, 14, 19, 25, 26, 33 are their values in ascending order. With $n = 5$ is obtained

$$f = \frac{n+1}{2} = 3$$

$$l = \frac{f(f-1)}{2} = 3$$

$$c_n = 2{,}2219 \frac{n}{n+1{,}4} = \frac{2{,}2219 \times 5}{6{,}4} = 1{,}736$$

and, since $l = 3$, it is necessary to pick the third smallest value from the ordered series of absolute deviations as Rousseeuw's Q_n. For this: $Q_n = 8$. Then $c_n Q_n = 1{,}736 \times 8 = 13{,}9$ is an unbiased estimate of σ.

NOTE If n is an even number then $f = (n/2) + 1$, e.g. if $n = 10$, then $f = (10/2) + 1 = 6$, and $l = (6 \times 5)/2 = 15$.

Annex V
(informative)

Indicators for Mandel's robust h- and k-statistics[2)]

Table V.1

p	$h_{5\,\%}$	$h_{1\,\%}$	$k_{5\,\%}$	$k_{1\,\%}$
8	1,98	3,23	1,78	2,60
9	2,11	3,38	1,79	2,59
10	1,98	2,99	1,81	2,59
11	2,04	3,08	1,82	2,59
12	1,97	2,90	1,83	2,57
13	2,00	2,93	1,84	2,57
14	1,97	2,83	1,85	2,57
15	1,98	2,85	1,86	2,57
16	1,96	2,77	1,86	2,57
17	1,97	2,78	1,87	2,57
18	1,96	2,74	1,87	2,57
19	1,97	2,76	1,88	2,57
20	1,96	2,71	1,88	2,57
21	1,96	2,72	1,89	2,56
22	1,96	2,69	1,89	2,56
23	1,95	2,69	1,89	2,56
24	1,95	2,67	1,90	2,56
25	1,95	2,68	1,90	2,56
26	1,95	2,67	1,90	2,56
27	1,95	2,66	1,90	2,56
28	1,95	2,66	1,90	2,56
29	1,95	2,65	1,91	2,56
30	1,95	2,65	1,91	2,56
31	1,95	2,63	1,91	2,56
32	1,95	2,63	1,91	2,56
33	1,95	2,63	1,91	2,56
34	1,95	2,63	1,91	2,56
35	1,95	2,63	1,92	2,56
36	1,95	2,63	1,92	2,56
37	1,95	2,63	1,92	2,56
38	1,95	2.63	1,92	2,56
39	1,95	2,63	1,92	2,56
40	1,95	2,63	1,92	2,56

2) The indicator values have been obtained in a simulation study with 1 million normal random samples for each number of laboratories, p.

Annex W
(informative)

Example: Determination of *E. coli*

In an interlaboratory study with 14 laboratories an alternative method for the determination of *E. coli* was compared with the reference method. Tables W.1, W.2 and W.3 present the measurement results (counts) at the three levels (low, medium and high contamination).

Table W.1 — Presentation of the results of the interlaboratory study at level $j = 1$ (low)

Laboratory	Reference method		Alternative method	
i	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	35	40	40	80
2	43	42	53	58
3	65	55	50	40
4	30	50	60	80
5	44	45	50	40
6	25	41	52	49
7	35	45	30	30
8	25	43	39	38
9	40	39	50	47
10	39	34	44	43
11	35	45	60	20
12	35	50	30	50
13	39	43	55	43
14	24	20	47	42

Table W.2 — Presentation of the results of the interlaboratory study at level $j = 2$ (medium)

Laboratory	Reference method		Alternative method	
i	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	390	510	550	520
2	620	550	670	630
3	380	400	350	460
4	440	360	500	570
5	480	380	540	560
6	490	600	620	610
7	360	330	190	410
8	360	400	480	400
9	420	510	570	520
10	440	400	490	290
11	590	420	480	460
12	430	480	590	470
13	430	480	500	480
14	450	360	420	410

Table W.3 — Presentation of the results of the interlaboratory study at level $j = 3$ (high)

Laboratory i	Reference method Duplicate 1	Reference method Duplicate 2	Alternative method Duplicate 1	Alternative method Duplicate 2
1	3 200	3 800	4 400	4 300
2	5 500	4 900	6 600	5 300
3	5 300	4 400	4 900	4 800
4	3 000	3 600	4 100	4 500
5	4 800	4 400	5 900	5 600
6	5 200	7 000	5 400	5 800
7	3 500	3 500	3 300	5 400
8	4 000	5 000	4 500	3 500
9	4 000	4 900	5 600	6 400
10	6 100	5 000	3 700	5 000
11	4 600	5 000	5 900	4 800
12	4 800	6 500	4 800	3 900
13	4 800	6 500	5 700	5 100
14	3 600	3 700	5 800	6 600

The application of the step by step procedure of 6.3.4 for the reference method, level $j = 1$ (low), is shown.

Step 1. Table W.4 is prepared. The logarithms of the counts of Table W.1, columns 2 and 3 are calculated and transferred into Table W.4, columns 2 and 3. The means and deviations (columns 4 to 6) are calculated.

Table W.4 — Measurement results (logarithms of counts), means and deviations for the reference method at level $j = 1$ (low)

1	2	3	4	5	6
Laboratory	Measurement		Mean	Deviation	
1	1,544	1,602	1,573	−0,029	0,029
2	1,633	1,623	1,628	0,005	−0,005
3	1,813	1,740	1,777	0,036	−0,036
4	1,477	1,699	1,588	−0,111	0,111
5	1,643	1,653	1,648	−0,005	0,005
6	1,398	1,613	1,505	−0,107	0,107
7	1,544	1,653	1,599	−0,055	0,055
8	1,398	1,633	1,516	−0,118	0,118
9	1,602	1,591	1,597	0,005	−0,005
10	1,591	1,531	1,561	0,030	−0,030
11	1,544	1,653	1,599	−0,055	0,055
12	1,544	1,699	1,622	−0,077	0,077
13	1,591	1,633	1,612	−0,021	0,021
14	1,380	1,301	1,341	0,040	−0,040

Step 2. For the $n = 2p = 2 \times 14 = 28$ deviations in Table W.4, columns 5 and 6, the values $c_{28} = 2{,}221\ 9 \times 28/(28 + 3{,}8) = 1{,}956$, and Q_n using the procedure described in Annex Q are determined. Each of the 28 deviation values is compared with the other 27 deviations to obtain a series of absolute differences, as illustrated below for the first seven deviations.

	–0,029 0	**0,005 1**	**0,036 3**	**–0,110 9**	**–0,0049**	**–0,1074**	**–0,0546**	**………**
–0,029 0		0,034 106	0,065 271	0,081 928	0,024 116	0,078 426	0,025 576	………
0,005 1			0,031 166	0,116 034	0,009 99	0,112 532	0,059 682	………
0,036 3				0,147 2	0,041 155	0,143 697	0,090 848	………
–0,110 9					0,106 044	0,003 502	0,056 352	………
–0,004 9						0,102 542	0,049 692	………
–0,107 4							0,052 85	………
–0,054 6								……...

Then all absolute difference values are ranked in ascending order. The lth lowest value in the ranked absolute differences is identified from $f = (n/2) + 1 = (28/2) + 1 = 15$ and $l = f(f - 1)/2 = 15 \times 14/2 = 105$. The value of Q_n is given by the 105th lowest value. In this example, $Q_n = 0{,}034\ 09$ and hence $Q_{\text{intra}} = 1{,}956 \times 0{,}034\ 09 = 0{,}066\ 70$.

Step 3. Again, according to Annex Q, with $n = p = 14$ and the 14 means of Table W.4, column 4, the values $c_{14} = 2{,}221\ 9 \times 14/(14 + 3{,}8) = 1{,}747\ 6$, $Q_n = 0{,}031\ 80$, and $Q_{\text{inter}} = 1{,}747\ 6 \times 0{,}031\ 80 = 0{,}055\ 57$ are obtained.

Step 4. The median of the means of Table W.4, column 4 is $m = 1{,}597\ 6$.

Step 5. The repeatability standard deviation is $s_r = \sqrt{2}\ Q_{\text{intra}} = 0{,}094\ 3$.

The coefficient of variation of repeatability is $C_{V,r} = s_r/m = 0{,}094\ 3/1{,}597\ 6 = 0{,}059\ 0 = 5{,}90\ \%$.

The repeatability limit is $r = 2{,}8 s_r = 2{,}8 \times 0{,}094\ 3 = 0{,}264$.

Step 6. The between laboratories standard deviation is $s_L = 0$ since $Q_{\text{inter}}^2 - Q_{\text{intra}}^2 \leq 0$.

The reproducibility standard deviation is $s_R = \sqrt{s_L^2 + s_r^2} = 0{,}094\ 3$.

The coefficient of variation of reproducibility is $C_{V,R} = s_R/m = 0{,}059\ 0 = 5{,}90\ \%$.

The reproducibility limit is $R = 2{,}8 s_R = 0{,}264$.

Step 7. Steps 1 to 6 are repeated for the alternative method.

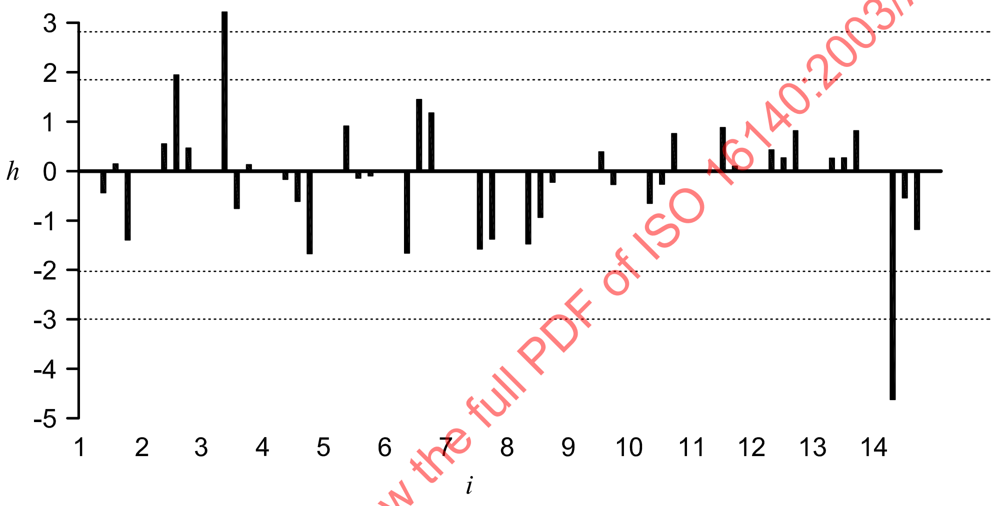
The calculations of steps 1 to 7 are also carried out for the other two levels, i.e. medium and high contamination; the results are combined in Table W.5.

The measurement results of the laboratories are scrutinized for consistency with Mandel's h- and k-values. For the reference method at level $j = 1$ (low) the h-values are calculated as differences between the means in Table W.4, column 4 and the median $m = 1{,}597\ 6$ (see step 4) divided by $Q_{\text{inter}} = 0{,}055\ 57$ (see step 3); the k-values are calculated as absolute values of the differences between the measurement results of Table W.4, columns 2 and 3 divided by $\sqrt{2} s_r = \sqrt{2} \times 0{,}094\ 3 = 0{,}133$. The h- and k-values for the other levels and the alternative method are calculated accordingly.

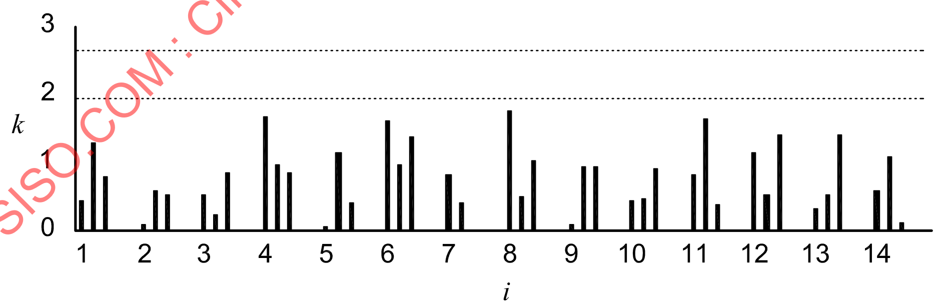
Figures W.1 and W.2 show the h and k plots for the reference method and the alternative method, respectively. Horizontal lines at the 5 % and 1 % significance indicators taken from Table V.1 are added. These graphs do not indicate remarkable inconsistencies between measurement results or laboratories.

Table W.5 — Results of the statistical analysis

Level	Reference method			Alternative method		
	median	repeatability s.d.	reproducibility s.d.	median	repeatability s.d.	reproducibility s.d.
1 (low)	1,597 6	0,094 3	0,094 3	1,650 5	0,091 3	0,116 4
2 (medium)	2,639 9	0,063 3	0,078 8	2,705 8	0,054 2	0,101 8
3 (high)	3,671 6	0,066 6	0,103 8	3,705 9	0,066 4	0,080 6



a)



b)

Key

h Mandel h-values (robust)

k Mandel k-values (robust)

i laboratory

Figure W.1 — Reference method, robust analysis