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Soil quality — Determination of abundance and activity of soil microflora using respiration curves

Qualité du sol — Détermination de l'abondance et de l'activité de la microflore du sol à l'aide de courbes de respiration

Reference number
ISO 17155:2002(E)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 17155 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Annex A of this International Standard is for information only.

Soil quality — Determination of abundance and activity of soil microflora using respiration curves

1 Scope

This International Standard specifies a test method for determining the activity of the active aerobic, heterotrophic microbial biomass in soils. This method is applicable to the monitoring of soil quality and to the evaluation of the ecotoxic potential of soils and soil materials. It is also applicable to soils that are contaminated experimentally in the field or in the laboratory (chemical testing) and for soils sampled along contamination gradients in the field.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14238:1997, *Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes*

3 Terms and definitions

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

3.1

basal respiration rate

R_B

constant mass of CO_2 released or mass of O_2 consumed per unit mass of soil per unit time without substrate addition

See Figure 1.

3.2

substrate-induced respiration

R_S

constant mass of CO_2 released or mass of O_2 consumed per unit mass of soil per unit time shortly after addition of a carbon substrate

See Figure 1.

NOTE If glucose is used as a carbon substrate, microbial biomass can be determined from the substrate-induced respiration rate (see Reference [1] in the Bibliography).

3.3

lag time

t_lag

time from the addition of a growth substrate until exponential growth starts

See Figure 1.

NOTE It reflects the vitality of the growing organisms (see Reference [2] in the Bibliography).

3.4

growth rate

μ

rate constant during exponential increase of the respiration rate

See Figure 1.

3.5

respiratory activation quotient

Q_R

basal respiration rate divided by substrate-induced respiration rate

$$Q_\mathrm{R} = R_\mathrm{B}/R_\mathrm{S}$$

3.6

time to the peak maximum

t_peakmax

time from addition of growth substrate to the maximum respiration rate

See Figure 1.

NOTE It reflects also the viability of the growing organisms.

3.7

cumulative CO_2 evolution or O_2 consumption

C_R

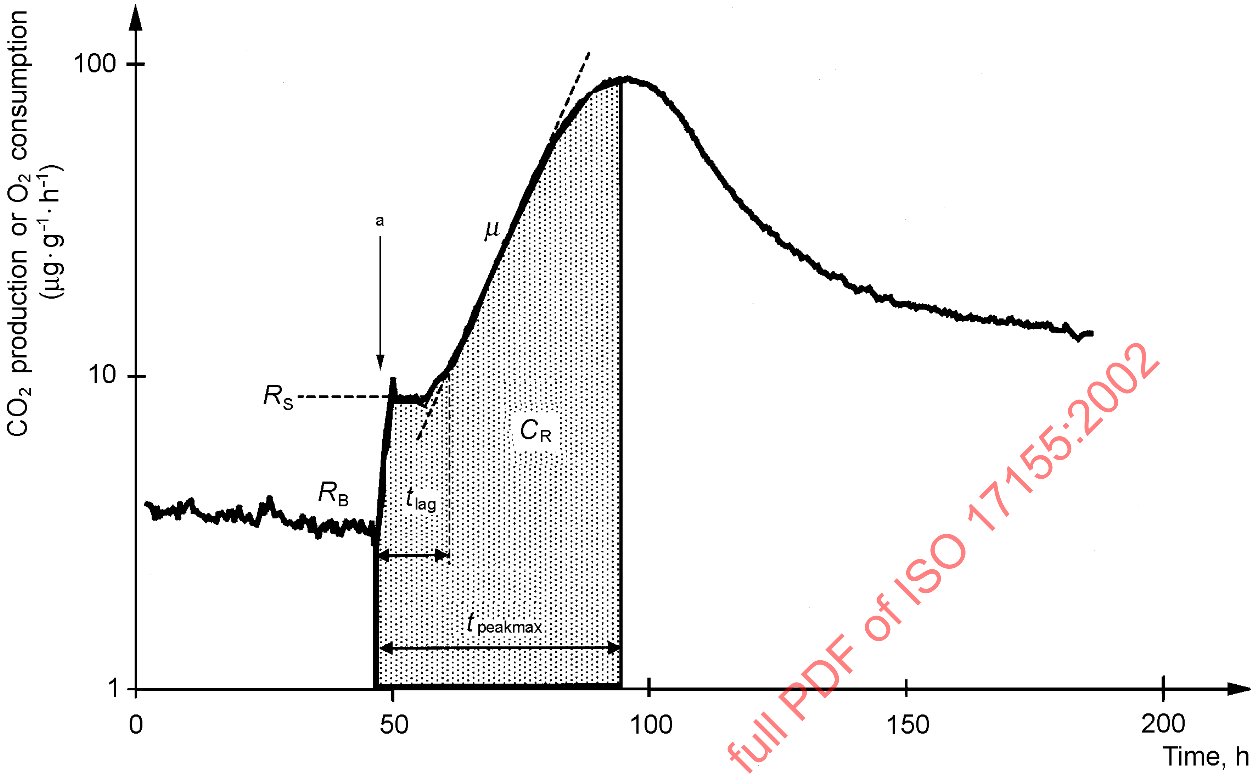
total area bounded by the soil respiration rate curve to the time axis from time of the addition of substrate to the time

See Figure 1.

3.8

soil material

material composed of excavated soil, dredged materials, manufactured soils, treated soils and fill materials



[a] Addition of substrate

Figure 1 — Soil respiration rate before and after addition of an easily degraded substrate

4 Principle

The CO_2 production or O_2 consumption (respiration rate) from unamended soils as well as the decomposition of an easily degraded substrate (glucose + ammonium + phosphate) is monitored regularly (at least every hour). Using the CO_2 production or O_2 consumption data, the different microbial parameters (basal respiration, substrate-induced respiration, lag time, respiratory activation quotient, t_{peakmax}, C_{R}) can be calculated.

5 Reagents

5.1 Glucose, $C_6H_{12}O_6$.

5.2 Potassium dihydrogen phosphate, KH_2PO_4.

5.3 Diammonium sulfate, $(NH_4)_2SO_4$.

5.4 Substrate, consisting of a mixture of 80 g of glucose (5.1), 13 g of diammonium sulfate (5.3) and 2 g of KH_2PO_4 (5.2) which is thoroughly ground and mixed in a mortar.

6 Apparatus

Ordinary laboratory equipment and

6.1 Respirometer for continuous measurement of CO_2 evolution or O_2 consumption, maintained at a constant temperature (preferably 20 °C). Suitable examples of equipment are given in ISO 16072[3].

7 Sampling

7.1 Sample quantities

Choose the size of the soil samples size with respect to the apparatus (6.1) used and on the organic matter content of the sample (7.1). Samples of organic horizons (e.g. mor layers) should not exceed 1 g of organic matter (see References [4] and [5]) in order to provide an optimal substrate/soil ratio (see 8.1). It is recommended to measure at least three replicates per sample.

7.2 Sampling and storage

The recommendations in ISO 10381-6 for the collection, handling and storage of soil samples shall be followed.

7.3 Soil sample characteristics

Soil samples generating soil respiration curves can be obtained from mineral soils, organic soils, polluted and unpolluted soils. Determine the following characteristics for each soil sample:

- particle size distribution in accordance with ISO 11277;
- water content in accordance with ISO 11465;
- water-holding capacity in accordance with annex A of ISO 14238:1997;
- pH in accordance with ISO 10390;
- organic matter content in accordance with ISO 10694.

8 Procedure

8.1 Test

Pre-incubate moist soil samples (preferably 40 % to 60 % of maximum water holding capacity or 0,01 MPa to 0,03 MPa suction pressure) at 20 °C for 3 d to 4 d before the beginning of the measurement. Measure the basal respiration of the sub-samples first. Measure the respiration rates until constant rates are obtained.

After measuring the basal respiration, add 0,2 g of the substrate (5.4) per gram of organic matter and mix homogeneously with a spatula into the soil samples. If the organic matter content is less than 5 % the substrate-to-soil ratio is 1 % [i.e. 1 g of substrate added per 100 g of soil (dry mass)].

Continue to measure the CO_2 evolution or O_2 consumption until the respiration rates decline (see Figure 1).

8.2 Influence of chemicals

The effect of chemicals on the soil microbial activity can be determined as follows. Using a range-finding test, determine the concentration range that chemicals would likely have an effect on this activity. Test a single, microbiologically active soil at five concentrations in a logarithmic series including a blank control in triplicate (for example 0, 1, 3,2, 10, 32, and 100 times the lowest concentration). Use the test procedure specified in 8.1. Using this simple test design, dose-response relationships can be established.

Before the start of the test, the test chemical may be added to the soil in one of the following manners:

- in an aqueous solution (depending on its solubility in water);
- in an organic solution using a water-miscible solvent (depending on the solubility in the solvent);
- mixed with a solid, e.g. coated on quartz sand (prior to mixing with the soil).

If the test chemical is added in the form of an organic solution, keep the amount of water-miscible solvent to the minimum ($< 1\,\%$) necessary for the application of the compound. Furthermore, take into account the possible toxicity and biodegradability of the solvent used.

NOTE Long-term effects of chemicals can be detected by using different incubation times (weeks or months).

9 Calculation

9.1 Microbial parameters

9.1.1 Basal respiration

Calculate the basal respiration (R_B) as the average of the hourly respiration rate during a stable period.

9.1.2 Substrate-induced respiration

Calculate the substrate-induced respiration (R_S) as the average of the values shortly after the substrate addition when the respiration is fairly constant after substrate addition. A minimum of 3 hourly measurements should be used to calculate the average.

9.1.3 Respiratory activation quotient

Calculate the respiratory activation quotient (Q_R) by dividing the basal respiration rate by the substrate-induced respiration rate.

$$Q_\text{R} = R_\text{B}/R_\text{S}$$

9.1.4 Lag time, growth rate and time to the peak maximum

Calculate the lag time (t_lag), growth rate (μ) and time to the peak maximum (t_peakmax) by making a plot of the logarithm of the respiration rate against time (see Figure 1). The exponential phase of the growth will then appear linear and a regression can be made giving the growth constant, μ, and the intercept with the y-axis. The lag time can be calculated as the time between substrate addition and the beginning of exponential growth (see Figure 1). t_peakmax can be calculated as the time between substrate addition and the maximum respiration rate.

NOTE A mathematical equation $\text{d}p/\text{d}t = r\text{e}^{\mu t} + K$ to which the data can be fitted was given by Stenström et al.[2] The respiration rate $\text{d}p/\text{d}t$ (where p is the product and $\text{d}p/\text{d}t$ is the rate of microbial product formation) after substrate addition represents the sum of the respiration rates of growing ($r\text{e}^{\mu t}$) and non-growing micro-organisms (K). According to the equation for substrate-induced respiration (SIR), $\text{SIR} = r + K$ at the time of substrate addition, where r is the initial respiration rate of growing micro-organisms and K the rate of non-growing micro-organisms.

9.1.5 Cumulative CO_2 evolution or O_2 consumption

The effect of a chemical on the lag time and on μ can be combined by determining the cumulative CO_2 evolution or O_2 consumption from the time point of the addition of substrate to the time point of the maximum respiration in the blank control (i.e. t_peakmax in Figure 1). It is essentially equivalent with the (linear) shaded surface area in Figure 1.

For each concentration, the cumulative CO_2 evolution or O_2 consumption (C_R) is measured to the t_peakmax in the control blank. A plot of C_R versus the logarithmic concentration of the test substance often gives an S-shaped curve from which EC_{10} and EC_{50} can be estimated.

NOTE This test is carried out under conditions stimulating microbial growth. However, a soil respiration inhibition test can also be carried out at low, growth-limiting concentrations of ^{14}C-labelled acetate followed by $^{14}CO_2$ determination (see Reference [9]).

9.2 Interpretation of data

9.2.1 Evaluation of the ecotoxic potential of soils

Polluted soils often show higher respiratory activation quotients and longer lag times and longer time to the peak maximum (t_{peakmax}) than unpolluted soils (see Figure 2). Moreover, polluted samples often do not show any logarithmic increase of respiration rates after addition of substrate and/or formation of double peaks. By comparing the parameters calculated in clause 9 with those obtained from unpolluted soils of similar physical and chemical properties, the contamination of soils or soil materials can be detected. Respiratory activation quotients, $Q_{\mathrm{R}} > 0{,}3$, lag times > 20 h and $t_{\mathrm{peakmax}} > 50$ h are indicative of polluted materials (see References [7] and [8] in the Bibliography).

In the case of double peaks, it is recommended to evaluate the growth rate, lag time, and t_{peakmax} for the second peak.

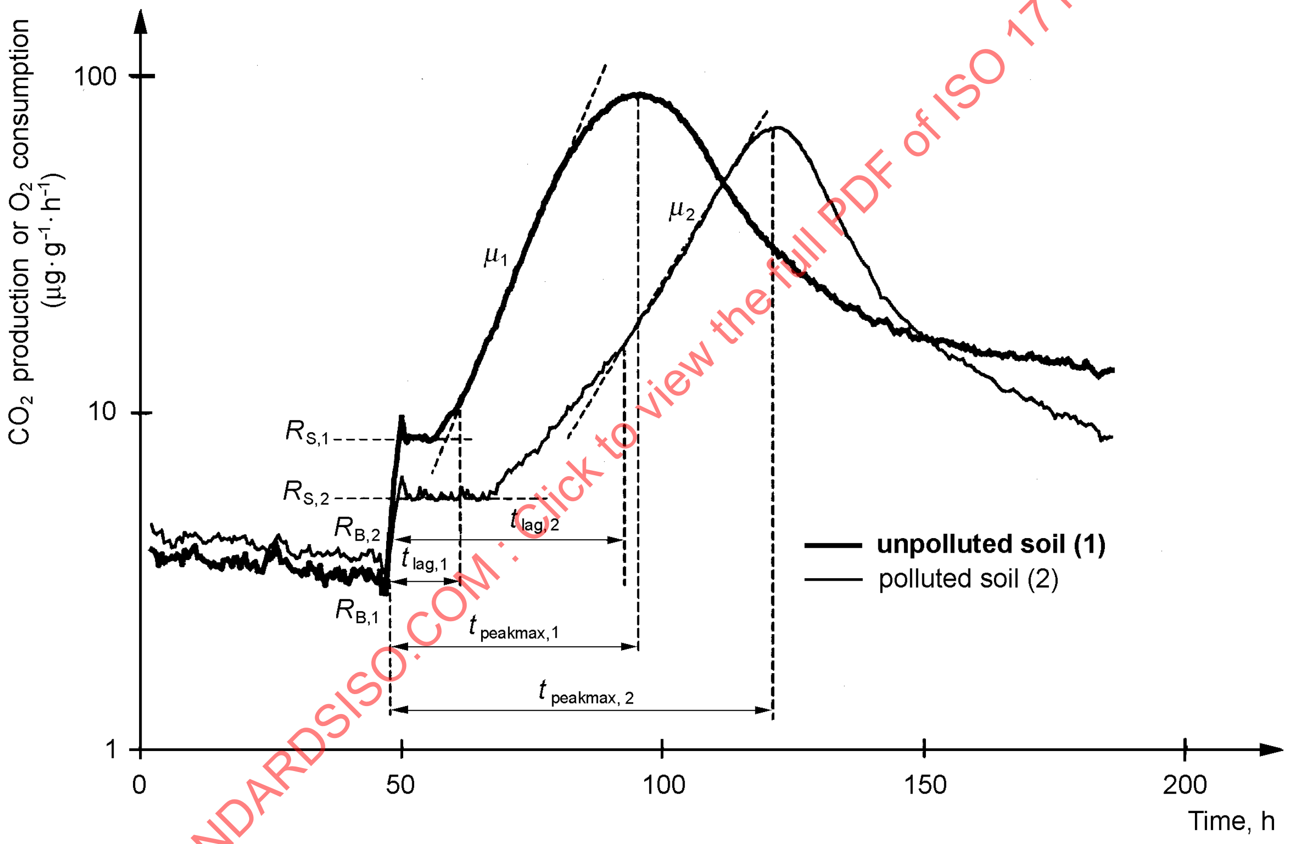


Figure 2 — Soil respiration curves of an unpolluted and a polluted soil

9.2.2 Additional criteria for the interpretation of the results from experimentally contaminated soils

Theoretically, when a chemical is experimentally added to soil, the microflora may respond in four different ways:

- Death: the substance is very toxic. The respiration decreases rapidly but if the toxic substance is removed, either by degradation or evaporation, the survivors can decompose the dead biomass and the respiration will temporarily be as high or higher than before the addition of the substance but the biomass will remain low for a considerable time (see Reference [10]).
- Intolerance: the toxicity of the test substance is moderate. Sensitive species are replaced by more resistant ones. The decomposition of soil organic matter becomes less efficient and less biomass is formed (see Reference [11]). The activity and vitality of the micro-organisms can possibly also be reduced.

- No observed effect: the toxicity of the test substance is small. If some species are affected they are replaced by others that are as effective as the original flora (see Reference [12]). CO_2 from the slow degradation of an organic test substance can possibly mask a reduced degradation of soil organic matter.
- Enhancement: the test substance is a suitable substrate for at least some of the soil organisms. Respiration is increased until the test substance is consumed (see Reference [13]). The biomass and vitality of the growing organisms are also increased.

The responses of intolerance or no observed effect to a chemical are the most likely to occur.

10 Test report

The test report should include the following information:

a) general:

  1) soil collection, treatment, incubation, including date collected, length of storage, temperature of storage;

  2) test substance: chemical identification data (chemical testing);

  3) soil characteristics (see also 7.3):

    - particle size distribution in accordance with ISO 11277;
    - water content in accordance with ISO 11465;
    - water-holding capacity in accordance with annex A of ISO 14238:1997;
    - pH in accordance with ISO 10390;
    - organic matter content in accordance with ISO 10694;

b) test conditions:

  1) date and place of sampling;

  2) date of start and end of the test;

  3) temperature;

  4) incubation time with the test substance before microbial measurements (chemical testing);

  5) concentrations tested or range of concentrations in test area compared to background levels (chemical testing);

  6) solvent used to add the test substance (chemical testing);

c) results:

  1) list of the microbial parameters for each sub-sample;

  2) mean values for each sample;

  3) plot of log of concentration of test substance against microbial parameters, EC_{10} and EC_{50} (chemical testing);

  4) linear regression of these relationships (chemical testing).

Annex A
(informative)

Results of an interlaboratory test carried out in Germany

An interlaboratory test using four different contaminated soils (see Table A.1) was carried out in Germany. Results obtained from CO_2 and O_2 measurements are given separately because in contaminated soils there is no constant ratio of CO_2/O_2. Outlier values were eliminated in accordance with to DIN 38402-42 (see Reference [14]).

Table A.1 — Chemical and physical properties and contaminant levels of soils

Properties and contaminant levels	**Soil sample**			
	1 loam	2 sand	3 sand	4 sand
WHC(max) [ml/kg]	511,8	347,7	241,8	268,5
pH	7,3	7,3	7,5	5,6
C_{org} [%]	4,7	8,5	5,9	1,7
N_t [%]	0,38	0,27	0,20	0,16
Σ Nitrotoluolenes [mg/kg]	336,3	n.d.	n.d.	n.d.
16 PAH [mg/kg]	77,0	60	21	2 268
Mineral oil [mg/kg]	$<$ 50,0	43 660,1	8 798,9	$<$ 50
As [mg/kg]	5,4	3,6	12,1	$<$ 3
Cod [mg/kg]	6,4	29,6	9,3	$<$ 3
Cr [mg/kg]	65,9	2 005,1	320,3	94,9
Cu [mg/kg]	104,4	10 921,3	367,8	10,0
Ni [mg/kg]	29,2	5 894,2	578,5	6,5
Pb [mg/kg]	820,8	1 082,2	547,5	10,3
Zn [mg/kg]	764,7	5 065,3	577,6	26,6

WHC(max): maximum water holding capacity
C_{org}: organic carbon content
N_t: total nitrogen content
n.d.: not determined

Table A.2 — Basal respiration rates (R_B)

Soil samples	**CO_2 measurement**				**O_2 measurement**			
	Mean values $\mu g \cdot g^{-1} \cdot h^{-1}$ CO_2	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]	Mean values $\mu g \cdot g^{-1} \cdot h^{-1}$ CO_2	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]
1	2,08	66	8	8	0,79	27	11	10
2	5,27	53	8	8	7,77	16	11	10
3	5,20	37	7	7	5,17	10	11	10
4	3,37	50	8	8	2,74	29	11	10

[a] Excluding those whose results were discarded as outliers.

Table A.3 — Substrate induced respiration (R_S)

Soil samples	**CO$_2$ measurement**				**O$_2$ measurement**			
	Mean values $\mu g \cdot g^{-1} \cdot h^{-1}$ CO$_2$	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]	Mean values $\mu g \cdot g^{-1} \cdot h^{-1}$ CO$_2$	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]
1	8,45	41	8	8	5,84	84	10	9
2	10,72	65	8	8	11,38	40	11	10
3	12,11	28	7	6	8,83	48	11	10
4	6,14	43	8	8	14,39	98	11	11

[a] Excluding those whose results were discarded as outliers.

Table A.4 — Respiratory activation quotient (Q_R)

Soil samples	**CO$_2$ measurement**				**O$_2$ measurement**			
	Mean values	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]	Mean values	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]
1	0,21	43	8	7	0,20	65	10	9
2	0,58	47	8	8	0,78	36	10	10
3	0,32	44	7	6	0,71	32	10	10
4	0,60	45	8	8	0,46	65	11	11

[a] Excluding those whose results were discarded as outliers.

Table A.5 — Lag time (t_{lag})

Soil samples	**CO$_2$ measurement**				**O$_2$ measurement**			
	Mean values h	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]	Mean values h	CV %	No. of participating labs	No. of laboratories whose results were accepted[a]
1	21	39	6	6	19	58	7	7
2	23	60	5	5	15	54	6	5
3	27	60	5	5	19	49	6	6
4	6	51	5	5	30	86	6	6

[a] Excluding those whose results were discarded as outliers.

Table A.6 — Time to the peak maximum (t_{peakmax})[a]

Soil samples	CO_2 measurement				O_2 measurement			
	Mean values h	CV %	No. of participating labs	No. of laboratories whose results were accepted[b]	Mean values h	CV %	No. of participating labs	No. of laboratories whose results were accepted[b]
1	55	38	7	7	65	23	7	7
2	71	26	6	6	69	43	7	7
3	61	23	7	7	54	43	6	6
4	47	37	7	7	60	16	7	7

[a] In cases where there were double peaks, only the second peak was considered.

[b] Excluding those whose results were discarded as outliers.