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IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[1)2)]

Scientific Division
Committee on Reference Systems for Enzymes (C-RSE)[3)]

Part 4. Reference Procedure for the Measurement of Catalytic Concentration of Alanine Aminotransferase

[L-Alanine: 2-Oxoglutarate Aminotransferase (ALT), EC 2.6.1.2]

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This paper is the fourth in a series dealing with reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C and the certification of reference preparations. Other parts deal with:

Part 1. The Concept of Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes; Part 2. Reference Procedure for the Measurement of Catalytic Concentration of Creatine Kinase; Part 3. Reference Procedure for the Measurement of Catalytic Concentration of Lactate Dehydrogenase; Part 5. Reference Procedure for the Measurement of Catalytic Concentration of Aspartate Aminotransferase; Part 6. Reference Procedure for the Measurement of Catalytic Concentration of γ-Glutamyltrans-

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ferase; Part 7. Certification of Four Reference Materials for the Determination of Enzymatic Activity of γ-Glutamyltransferase, Lactate Dehydrogenase, Alanine Aminotransferase and Creatine Kinase at 37 °C.

A document describing the determination of preliminary upper reference limits is also in preparation. The procedure described here is deduced from the previously described 30 °C IFCC reference method (1). Differences are tabulated and commented on in Appendix 2. Clin Chem Lab Med 2002; 40(7):718–724

Key words: IFCC reference procedure; Alanine aminotransferase; Preliminary upper reference limit.

Abbreviations: ALT, alanine aminotransferase; LDH, lactate dehydrogenase; NAD, β-nicotinamide adenine dinucleotide; NADH, β-nicotinamide adenine dinucleotide, reduced form.

Reaction Principle

L-Alanine + 2-Oxoglutarate -ALT→ Pyruvate + L-Glutamate
Pyruvate + NADH + H^+ -LDH*→ Lactate + NAD^+
*Lactate dehydrogenase (LDH, EC 1.1.1.27)

Specimens

Calibration materials, control specimens and human sera.

Measurement Conditions

Concentrations in the final reaction mixture and the measurement conditions are listed in Tables 1 and 2.

Table 1 Concentrations in the final complete reaction mixture for the measurement of ALT.

Tris(hydroxymethyl)aminomethane	100 mmol/l
pH (37 °C)	7.15±0.05*
L-Alanine	500 mmol/l
NADH	0.18 mmol/l
Pyridoxal-5′-phosphate	0.1 mmol/l
Lactate dehydrogenase (37 °C)	28.3 µkat/l (1700 U/l)
2-Oxoglutarate	15 mmol/l
Volume fraction of sample	0.0833 (1:12)

*expanded (k=2) combined uncertainty

Table 2 Conditions for the measurement of ALT.

Temperature	37.0 °C±0.1 °C*
Wave length	339 nm±1 nm *
Band width	≤2 nm
Light path	10.00 mm±0.01 mm*
Incubation time	300 s
Delay time	90 s
Measurement interval	180 s
Readings (measurement points)	≥6

*expanded (k=2) combined uncertainty

Reagents

1. Tris(hydroxymethyl)aminomethane (Tris) ($C_4H_{11}NO_3$), M_r=121.1
2. L-Alanine, free acid ($C_3H_7NO_2$), M_r=89.09
3. 2-Oxoglutaric acid, crystallized disodium salt, dihydrate ($C_5H_4O_5Na_2 \cdot 2\ H_2O$), M_r=226.1
4. β-Nicotinamide adenine dinucleotide, reduced form (NADH) ($C_{21}H_{27}N_7O_{14}P_2Na_2$), disodium salt, M_r=709.4
   Pyridoxal-5′-phosphoric acid, monohydrate ($C_8H_{10}O_6NP \cdot H_2O$), M_r=265.2
5. Lactate dehydrogenase (LDH, EC 1.1.1.27), from pig skeletal muscle, in glycerol
6. Sodium chloride (NaCl), M_r=58.44
7. Sodium azide (NaN_3), M_r=65.01
8. Hydrochloric acid (HCl), M_r=36.46, 1 mol/l
9. Bovine serum albumin, Fraction V, M_r=68 000

Note: Ammonium sulphate suspension of the LDH reagent enzyme may not be used due to glutamate dehydrogenase activity in the sample.
Note: The reagent enzyme preparation (LDH and bovine serum albumin) must be free from glutamate dehydrogenase and ALT. The absence of these contaminants must be declared by the manufacturer or experimentally investigated in the reference laboratory.
Note: Contamination of 2-oxoglutarate by pyruvate leads to a consumption of NADH and decreases the initial absorbance of the final complete reaction mixture. Reagents of the highest purity must be used. If a chemical is suspected of containing impurities affecting the catalytic activity of the analyte, further investigations must be performed, *e.g.* comparisons with products from different manufacturers and different lots. It is recommended to use reagents which have already tested and approved in comparisons.

Charts for the Adjustment and the Control of the pH Values (Procedure for the Adjustment of pH Values at Temperatures Diverging from 37 °C)

Both the thermometer and the pH electrode are suspended in the mixed solution simultaneously. The stirred solution is then titrated to the pH value listed in the chart for the actually measured temperature. The speed of agitation should be the same during the calibration, the control and the adjustment of the pH value. The pH electrode should be positioned in the centre of the stirred solution.

The fact that the temperature can change during the titration must be taken into account. For this reason, the temperature in the proximity of the target value should be controlled again and the target pH value corrected according to Tables 3 and 4, if necessary. The same applies to the adjustment of the temperature compensation of the pH meter.

Preparation of Solutions

The given mass of the compounds for the preparation of solutions refers to 100% content. If the content of the reagent chemical employed is less (*e.g.* yz %), the amount equivalent to the given mass is calculated by the use of a factor: $F_{content}$=100/yz.

Highly purified water with a quality comparable to bi-distilled water (conductivity <2 µS/cm, pH 6–7, silicate <0.1 mg/l) shall be used for the preparation of the reagent solutions. The expanded (k=2) combined uncertainty (normally distributed) of each weighing procedure (including the uncertainty of the purity of the substance) shall be ≤1.5%.

Solution 1

1.47 g (121.2 mmol/l) Tris
5.61 g (630.0 mmol/l) L-Alanine, free acid
0.052 g (8.00 mmol/l) Sodium azide

Table 3 Dependence of the pH value of Solution 1 upon temperature.

Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH
15.00	7.758	23.50	7.511	32.00	7.279
15.25	7.751	23.75	7.504	32.25	7.273
15.50	7.743	24.00	7.497	32.50	7.266
15.75	7.736	24.25	7.490	32.75	7.259
16.00	7.728	24.50	7.483	33.00	7.253
16.25	7.721	24.75	7.476	33.25	7.246
16.50	7.714	25.00	7.469	33.50	7.240
16.75	7.706	25.25	7.462	33.75	7.233
17.00	7.699	25.50	7.455	34.00	7.227
17.25	7.691	25.75	7.448	34.25	7.220
17.50	7.684	26.00	7.441	34.50	7.214
17.75	7.677	26.25	7.435	34.75	7.207
18.00	7.669	26.50	7.428	35.00	7.201
18.25	7.662	26.75	7.421	35.25	7.194
18.50	7.655	27.00	7.414	35.50	7.188
18.75	7.647	27.25	7.407	35.75	7.182
19.00	7.640	27.50	7.400	36.00	7.175
19.25	7.633	27.75	7.393	36.25	7.169
19.50	7.626	28.00	7.387	36.50	7.162
19.75	7.618	28.25	7.380	36.75	7.156
20.00	7.611	28.50	7.373	37.00	7.150
20.25	7.604	28.75	7.366	37.25	7.143
20.50	7.597	29.00	7.359	37.50	7.137
20.75	7.590	29.25	7.353	37.75	7.131
21.00	7.582	29.50	7.346	38.00	7.124
21.25	7.575	29.75	7.339	38.25	7.118
21.50	7.568	30.00	7.332	38.50	7.112
21.75	7.561	30.25	7.326	38.75	7.105
22.00	7.554	30.50	7.319	39.00	7.099
22.25	7.547	30.75	7.312	39.25	7.093
22.50	7.540	31.00	7.306	39.50	7.087
22.75	7.532	31.25	7.299	39.75	7.081
23.00	7.525	31.50	7.292	40.00	7.074
23.25	7.518	31.75	7.286		

Table 4 Dependence of the pH value of Solution 2 upon temperature.

Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH
15.00	7.758	23.50	7.508	32.00	7.277
15.25	7.750	23.75	7.501	32.25	7.271
15.50	7.743	24.00	7.494	32.50	7.264
15.75	7.735	24.25	7.487	32.75	7.258
16.00	7.728	24.50	7.480	33.00	7.251
16.25	7.720	24.75	7.473	33.25	7.245
16.50	7.713	25.00	7.466	33.50	7.238
16.75	7.705	25.25	7.459	33.75	7.232
17.00	7.698	25.50	7.452	34.00	7.225
17.25	7.690	25.75	7.445	34.25	7.219
17.50	7.683	26.00	7.438	34.50	7.213
17.75	7.675	26.25	7.432	34.75	7.206
18.00	7.668	26.50	7.425	35.00	7.200
18.25	7.660	26.75	7.418	35.25	7.194
18.50	7.653	27.00	7.411	35.50	7.187
18.75	7.646	27.25	7.404	35.75	7.181
19.00	7.638	27.50	7.397	36.00	7.175
19.25	7.631	27.75	7.390	36.25	7.168
19.50	7.624	28.00	7.384	36.50	7.162
19.75	7.616	28.25	7.377	36.75	7.156
20.00	7.609	28.50	7.370	37.00	7.150
20.25	7.602	28.75	7.363	37.25	7.143
20.50	7.594	29.00	7.357	37.50	7.137
20.75	7.587	29.25	7.350	37.75	7.131
21.00	7.580	29.50	7.343	38.00	7.125
21.25	7.573	29.75	7.337	38.25	7.119
21.50	7.565	30.00	7.330	38.50	7.113
21.75	7.558	30.25	7.323	38.75	7.107
22.00	7.551	30.50	7.317	39.00	7.101
22.25	7.544	30.75	7.310	39.25	7.094
22.50	7.537	31.00	7.303	39.50	7.088
22.75	7.530	31.25	7.297	39.75	7.082
23.00	7.523	31.50	7.290	40.00	7.076
23.25	7.515	31.75	7.284		

– Dissolve in about 80 ml water.
– Adjust pH (37 °C) 7.15 with 1 mol/l hydrochloric acid.
– Transfer to a 100 ml volumetric flask.
– Equilibrate the volumetric flask and water to 20 °C.
– Fill the water (20 °C) up to the calibration mark of the volumetric flask.

Stability at 2 °C – 8 °C: 3 months

Solution 2

1.47 g (121.2 mmol/l) Tris
0.052 g (8.00 mmol/l) Sodium azide
– Dissolve in about 80 ml water.
– Adjust pH (37 °C) 7.15 with 1 mol/l hydrochloric acid.
– Transfer to a 100 ml volumetric flask.
– Equilibrate the volumetric flask and water to 20 °C.
– Fill the water (20 °C) up to the calibration mark of the volumetric flask.

Stability at 2 °C – 8 °C: 3 months

Solution 3

16.7 mg (6.300 mmol/l) Pyridoxal-5′-phosphoric acid, monohydrate
– Dissolve in about 6 ml Solution 2.
– Transfer to a 10 ml volumetric flask.
– Equilibrate volumetric flask and Solution 2 to 20 °C.
– Fill Solution 2 (20 °C) up to the calibration mark of the volumetric flask.
– Store protected from light (*e.g.* in a brown bottle).
Stability at 2 °C – 8 °C: 1 week

Solution 4

16.1 mg (11.34 mmol/l) NADH, disodium salt
– Dissolve in 2.00 ml Solution 2.
– Store protected from light (*e.g.* in a brown bottle).
Stability at 2 °C – 8 °C: 1 week

Diluent for reagent enzymes

1.20 g Bovine serum albumin
0.90 g (154 mmol/l) NaCl
– Dissolve in about 80 ml water.
– Transfer to a 100 ml volumetric flask.
– Equilibrate the volumetric flask and water to 20 °C.
– Fill the water (20 °C) up to the calibration mark of the volumetric flask.
Stability at 2 °C – 8 °C: at least 1 month

Solution 5

Lactate dehydrogenase solution [3.57 mkat/l (214 kU/l) at 37 °C]
– Dilute the LDH stock solution with diluent for reagent enzymes (see above) so that the dilution exhibits a catalytic LDH concentration of 3.57 mkat/l (214 kU/l) at 37 °C.
Example: LDH_{stock}: catalytic LDH concentration of the enzyme stock solution in mkat/l, see Appendix 1; $V_{diluent}$: volume of diluent for reagent enzymes for the dilution of the LDH stock solution. $V_{diluent}$=0.1 (LDH_{stock}–3.57)/3.57. Add to 0.1 ml enzyme stock solution the volume (ml) of $V_{diluent}$.
Stability at 4 °C: at least 2 days

Reaction solution

10.0 ml Solution 1
0.200 ml Solution 3
0.200 ml Solution 4
0.100 ml Solution 5
– Mix thoroughly and store light protected.
Stability at 2 °C – 8 °C: 1 day

Start reagent solution

0.407 g (180.0 mmol/l) 2-Oxoglutaric acid, disodium salt, dihydrate
– Dissolve in about 6 ml water.
– Transfer to a 10 ml volumetric flask.
– Equilibrate the volumetric flask and water to 20 °C.
– Fill the water (20 °C) up to the calibration mark of the volumetric flask.
Stability at 2 °C – 8 °C: 1 week

Measurement Procedure

Equilibrate only an adequate volume (~0.4 ml) of the start reagent solution at 37 °C in preparation for the measurement procedure. The remaining volume of the start reagent solution should be stored at 2 °C – 8 °C.

Pipette the following volumes one after another into the cuvette as listed in Table 5.

Table 5 Analytical system for the measurement of ALT.

2.000 ml	Reaction solution *Equilibrate to 37.0 °C.*
0.200 ml	Sample *Mix thoroughly and incubate for 300 s. At the end of the incubation time, the temperature of the solution in the cuvette shall have reached 37.0 °C.*
0.200 ml	Start reagent solution *Mix thoroughly, wait 90 s and monitor time and absorbance for additional 180 s.*

The expanded (k=2) combined uncertainty (normally distributed) of the kinetic photometric measurement shall not exceed 1%. (This uncertainty does not include the uncertainty of the wave length adjustment.)

The expanded (k=2) combined uncertainty (normally distributed) of the volume fraction of sample shall be ≤1%.

Reagent blank rate

To determine the reagent blank rate, the specimen is replaced by 9 g/l (154 mmol/l) sodium chloride solution. The measurement procedure is then carried out as described above. If the absolute reagent blank rate exceeds 3.3×10^{-5} s^{-1} (0.002 min^{-1}) or has a reverse direction, the measurements must be repeated and if necessary the reaction solution must be discarded.

Sample blank rate

For the determination of the sample blank rate, the start reagent solution is replaced by 9 g/l (154 mmol/l) sodium chloride solution. The measurement procedure is then carried out as described above.
Note: The sample blank rate is determined and documented but not taken into account for calculation of the catalytic concentration of ALT in control sera and calibrators. In case that the value of the sample blank rate exceeds 1% of total ALT, a warning that the respective material is not appropriate for calibration should be issued.
Note: The reagent blank rate for the sample blank rate is determined by replacing the start reagent solution **and** the sample by 9 g/l (154 mmol/l) sodium chloride solution.

Upper limit of the measurement range

If the change of absorbance exceeds 0.0025 s^{-1} (0.15 min^{-1}) in the measurement interval, an analytical portion of the sample must be diluted with 9 g/l (154 mmol/l) sodium chloride solution and the measurement procedure must be repeated with the diluted specimen. The obtained value must then be multiplied by the corresponding factor of the dilution.

Sources of error

High pyruvate concentrations in the sample lead to high NADH consumption during the incubation period. This can reduce the upper limit of the measurement range and considerably lower the results of analyses.

Calculation

The temporal change of absorbance (s^{-1}) is calculated with the analysis of regression (method of the least squares). After subtraction of the reagent blank rate the corrected change of absorbance is multiplied by the factor:

F = 1905 (measurement at 339 nm, ε_{339}(NADH) = 630 m^2/mol)

The catalytic concentration of ALT is calculated in µkat/l.

$\Delta A/\Delta t_{ALT}$: change of absorbance (in s^{-1}) after correction of the reagent blank rate

b_{ALT}: catalytic concentration of ALT

$b_{ALT}=1905\ \Delta A/\Delta t_{ALT}$

The catalytic concentration in µkat/l can be converted to U/l by multiplication by the factor f=60.

Preliminary Upper Reference Limits

The preliminary upper reference limits for adults (≥17 years) were investigated separately for men (n=422) and women (n=411).

Gender	Upper reference limit* (and 90% confidence interval)
Women	0.56 µkat/l (0.51 µkat/l – 0.60 µkat/l)
Men	0.74 µkat/l (0.71 µkat/l – 0.77 µkat/l)

Gender	Reference limit* (and 90% confidence interval)
Women	34 U/l (31 U/l – 36 U/l)
Men	45 U/l (42 U/l – 45 U/l)

*The upper reference limits are the 97.5th percentiles of the reference collectives. Inside brackets are the 90% confidence intervals of the 97.5th percentiles.

Appendix 1: Determination of the Catalytic Concentration of LDH in the Enzyme Stock Solution

Additional reagents

Pyruvic acid, monosodium salt ($C_3H_3O_3Na$), M_r=110.0

Measurement conditions

Concentrations in the reaction mixture and measurement conditions are listed in Tables 6 and 7.

Table 6 Concentrations in the final complete reaction mixture for the measurement of LDH.

Tris(hydroxymethyl)aminomethane	100 mmol/l
pH (37 °C)	7.15±0.05*
L-Alanine	500 mmol/l
NADH	0.18 mmol/l
Pyridoxal-5′-phosphate	0.1 mmol/l
Pyruvate	3 mmol/l
Volume fraction of sample	0.0833 (1:12)

*expanded (k=2) combined uncertainty

Table 7 Conditions for the measurement of LDH.

Temperature	37.0 °C±0.1 °C*
Wave length	339 nm±1 nm*
Band width	≤2 nm
Light path	10.00 mm±0.01 mm*
Incubation time	30 s
Delay time	30 s
Measurement interval	90 s
Readings (measurement points)	≥6

*expanded (k=2) combined uncertainty

Reaction solution

10.0 ml Solution 1
0.200 ml Solution 3
0.200 ml Solution 4
0.100 ml water
– Mix thoroughly and store protected from light.
Stability at 2 °C – 8 °C: 1 day

Start reagent solution

0.0990 g (36.00 mmol/l) Pyruvic acid, monosodium salt
– Dissolve in about 6 ml water.
– Transfer to a 25 ml volumetric flask.
– Equilibrate the volumetric flask and water to 20 °C.
– Fill the water (20 °C) up to the calibration mark of the volumetric flask.
Stability at 2 °C – 8 °C: 1 day

Dilution of the enzyme stock solution (immediately before use)

Step 1: Add 0.050 ml enzyme stock solution to 10.0 ml diluent for reagent enzymes and mix thoroughly. Step 2: Add 0.050 ml of the final solution from step 1 to 10.0 ml diluent for reagent enzymes and mix thoroughly.

Note: The catalytic concentration of the LDH stock solution may necessitate dilutions different from the above-described procedure. This requires respective modification of the dilution factor ($F_{dilution}$).

Measurement procedure

Equilibrate only an adequate volume (~0.4 ml) of the start reagent solution at 37 °C in preparation for the measurement procedure. The remaining volume of the start reagent solution should be stored at 2 °C – 8 °C.

Pipette the volumes one after another into the cuvette as listed in Table 8.

Table 8 Analytical system for the measurement of LDH.

2.000 ml	Reaction solution *Equilibrate to 37.0 °C.*
0.200 ml	LDH solution Step 2 *Mix thoroughly and incubate for 30 s. At the end of the incubation time, the temperature of the solution in the cuvette shall have reached 37.0 °C.*
0.200 ml	Start reagent solution *Mix thoroughly, wait 30 s and monitor time and absorbance for additional 90 s.*

To determine the reagent blank rate, the volume of the diluted enzyme stock solution is replaced by 9 g/l (154 mmol/l) sodium chloride solution. The measurement procedure is then carried out as described above.

Calculation

The calculation is the same as the calculation for the catalytic concentration of ALT. The result is the catalytic LDH concentration in the final solution of Step 2. For calculation of the catalytic LDH concentration in the enzyme stock solution (LDH_{stock}) this result must be multiplied by the dilution factor:

$F_{dilution}$=40401

Calculation:

$\Delta A/\Delta t_{LDH}$: change of absorbance (in s^{-1}) in the reaction mixture after subtraction of the reagent blank rate

$LDH_{stock}=1905 \cdot 40401 \cdot \Delta A/\Delta t_{LDH}$

The catalytic concentration in µkat/l can be converted to kU/l by multiplication by the factor f=0.06.

Appendix 2: Changes in the Reference Procedure for Measurements at 37 °C Compared with the Reference Method for Measurements at 30 °C as Described in the Original IFCC Document

The primary reference procedure is deduced from the IFCC reference method (1) which provides optimised conditions for the measurement of catalytic activity concentrations of ALT. The measurement temperature of 37 °C instead of 30 °C requires only minimal changes of certain measurement parameters to retain the optimum measurement conditions. The modifications are listed and commented on in Table 9. Furthermore, if in comparison to the 30 °C reference method a more accurate specification has become necessary for improving the high standardization of the measurements, it is also described here.

Table 9 Comparison of the IFCC methods for the measurement temperatures of 30 °C and 37 °C.

37 °C Reference procedure	30 °C Reference method	Comment
Specimen of investigation		
Calibration materials, control specimens and human sera	Human sera	The reference procedure will be used primarily for the investigation of calibration materials and control specimens.
pH value		
The pH optimum is 7.15	The pH optimum is 7.30	The shift of the pH optimum with the temperature coincidentally agrees with the shift of the pK value of the buffer. Therefore, the same reagent solution can be used at 30 °C and 37 °C.
Uncertainty of the pH value adjustment		
ΔpH ± 0.05	Not specified	
Uncertainty of the measurement temperature adjustment		
Uncertainty ≤0.1 °C (k=2)	Bias: less ± 0.05 °C Imprecision: less ± 0.1 °C	High quality spectrophotometer with devices for temperature adjustment and control provide an uncertainty (k=2) of the temperature ≤0.1 °C.
Incubation time		
300 s	At least 600 s	A time interval of 300 s is sufficient to saturate the ALT with pyridoxal phosphate at 37 °C.
Measurement interval		
180 s	At least 300 s	Higher signals at 37 °C allow shortening of the measurement time without enlarging the imprecision.

Catalytic LDH concentration		
28.3 µkat/l (1700 U/l)	20 µkat/l (1200 U/l)	Same amount of LDH for 30 °C and 37 °C. The higher catalytic concentration is due to the higher temperature.
Start reagent solution		
2-Oxoglutaric acid, disodium salt in water, contains no Tris. Therefore, higher Tris concentrations are necessary in Solution 1 and Solution 2. No pH adjustment is prescribed.	2-Oxoglutaric acid, free acid, contains Tris in water. The pH adjustment is prescribed.	The same composition as described for AST is used. The aqueous disodium salt solution has a pH at 25 °C of about 7.5 and practically no buffer capacity.
Diluent for the reagent enzyme		
Bovine serum albumin and sodium chloride in water	Glycerol/water	Instability of LDH was observed in some glycerol-water mixtures.
Sample blank rate		
Not taken into account	Subtraction	Usually, sample blank rates are not subtracted in routine procedures. Therefore, the assigned values in calibrators and control materials are only useful for routine methods, if they contain the sample blank rate value.
Volumes of the reagent solutions		
100 ml Tris/Hydrochloride 10.5 ml Reaction solution 10 ml Start reagent solution	200 ml Tris/Hydrochloride 105 ml Reaction solution 100 ml Start reagent solution	There is no need to prepare large volumes of reagent solutions, because the reagent solutions are prepared freshly for each campaign.
Temperature of the start reagent solution before use		
Before use the start reagent solution should have 37 °C.	No information about the temperature	The use of the Start reagent solution with ambient temperature decreases the temperature in the cuvette.
Collection of data		
Number of readings ≥6	Continuous monitoring of the change of absorbance	Modern spectrophotometers employ digital data processing. Several readings ≥ 6 should ensure a sufficient precision of the measurement results. Devices for a continuous monitoring are no longer in use.
Determination of the slope (time vs. absorbance)		
Regression analysis of the method of least squares	No information	A well-defined statistical method is necessary to ensure the reproducibility of the calculation of the slope.
Reference range		
Women ≤0.57 µkat/l (≤34 U/l) Men ≤0.77 µkat/l (≤45 U/l)	Healthy, young adults: 0.08 to 0.58 µkat/l	The reference values for women and men were investigated separately.

References

1. Bergmeyer HU, Hørder M, Rej R. International Federation of Clinical Chemistry (IFCC). Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 3. IFCC method for alanine aminotransferase. J Clin Chem Clin Biochem 1986; 24:481–95.