ENZYMATIC POTASSIUM
Insert

Ref.: 125

Intended use . System for quantitative determination of potassium ion in serum samples by kinetic enzymatic reaction.

[For in vitro diagnostic use.]

Test principle . Potassium is determined via enzymatic reaction, in which phosphoenolpyruvate is converted to pyruvate by the action of a potassium-dependent pyruvate kinase. The pyruvate produced is converted to lactate in presence of NADH (nicotinamide dinucleotide), in a reaction which is catalyzed by lactate dehydrogenase. The oxidation of NADH to NAD and subsequent decrease in optic density at 380 nm is proportional to the amount of potassium in sample.

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow[\text{Pyruvate kinase}]{K^+} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow[\text{Lactate dehydrogenase}]{} \text{Lactate} + \text{NAD}$$

Summary . The Enzymatic Potassium Labtest method was developed using the specificity of the potassium-depended pyruvate kinase, being a handful alternative to flame photometry and ion-selective electrode (ISE) methodologies, which require specific equipment.

The reaction components are distributed in two reagents that are ready to use, which makes the liquid presentation more stable. The great analytical specificity, which is easy to use in automated analyzers capable of measuring absorbance at 380 nm, allows the user to measure the potassium ion concentration along with other biochemical routine exams, making the analytical process practical and agile.

Methodology . Enzymatic reaction

Reagents

1. R 1 - Reagent 1 - Ready to use. Store at 2 - 8 ºC.
Lactate dehydrogenase <50 KU/L; Phosphoenolpyruvate <100 mmol/L; analogue NADH <10 mmol/L; ADP <100 mmol/L, and lithium azide <0.095%.

2. R 2 - Reagent 2 - Ready to use. Store at 2 - 8 ºC.
Pyruvate kinase <50 KU/L, and lithium azide <0.095%.

3. CAL 1 - Calibrator 1 - Ready to use. Store at 2 - 8 ºC.
Check the calibrator concentration on the bottle label.
Liquid preparation containing potassium ions in buffered solution 50 mM pH 7.4, and sodium azide <0.095%.

4. CAL 2 - Calibrator 2 - Ready to use. Store at 2 - 8 ºC.
Check the calibrator concentration on the bottle label.
Liquid preparation containing potassium ions in buffered solution 50 mM pH 7.4, and sodium azide <0.095%.

The reagents must be kept out of their storage temperature only for the time necessary to get the volume that will be used in tests.

Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label. Microbial or chemical contamination may decrease reagent stability.

Precautions and warnings

Ensure the calibrators are at room temperature prior to being used. Homogenize the calibrators gently before use.

Ensure the potassium determination be made after the sodium determination when both analytes are determined for the same sample.

It is recommended to insert a probe wash step in the automated analyzer protocol when potassium is being determined, so as to reduce cross contamination caused by dragging of reagents.

The presence of air bubbles in reagents, calibrators, or in any sample during the test execution is a common cause of error in analyte determination.

Do not mix reagents from different lots.

The usual security cares should be applied to the reagent handling. They must not be pipetted by mouth aspiration. Avoid ingestion and in case of contact with eyes, wash them with plenty of water and seek medical help.

The reagents and calibrators contain azide as preservative. Avoid ingestion. In case of contact with eyes, immediately flush eyes with plenty of water and get medical assistance.
Azide may react with lead and copper plumbing and yield highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Materials required not provided

1. Analyzer capable of measuring absorbance accurately at 380 nm.
2. Qualitrol H Labtest control series.
3. Water bath kept constantly at 37 ºC.
4. Pipets to measure samples and reagents.
5. Timer.

Labtest

Specimen collection and preparation

Use only serum. Do not use hemolysed samples. The analyte is stable for up to 5 days at 2 - 8 ºC and up to 12 months at -20 ºC, when stored in a tube proper for sample freezing.

Ensure all samples are thawed and properly homogenized prior to being used. Do not use samples with signs of contamination or samples which underwent repeated freeze and thaw cycles.

A Standard Operating Procedure (SOP) must be created to establish adequate procedures for sample collection, preparation, and storage. The errors due to bad sampling can be more damaging than the ones which may occur during the analytical procedure.

Since no known test method can offer complete assurance that human blood samples will not transmit infectious diseases, all blood samples should be considered potentially infectious and handled accordingly.

Disposal of all biological waste material should be in accordance with local guidelines.

Interference

Sodium ions up to 150 mM, conjugated bilirubin up to 20 mg/dL, unconjugated bilirubin up to 15 mg/dL, ascorbic acid up to 10 mM and triglycerides up to 1000 mg/dL do not interfere significantly in the reaction.

Procedure

Manual and semi-automated systems

1. In a tube named either Test, Calibrator 1 or Calibrator 2, pipet:

	Calibrator 1	Calibrator 2	Test
Reagent 1	0,600 mL	0,600 mL	0,600 mL
Sample	0,015 mL	0,015 mL	0,015 mL

2. Homogenize the tube and incubate it in a water bath at 37 ºC ± 0,2 ºC for 5 minutes.

3. Adjust the photometer blank using deionized water. Add:

Reagent 2	0,150 mL	0,150 mL	0,150 mL

4. Homogenize the tube and transfer its content immediately to the thermostated cuvette at 37 ºC ± 0.2 ºC. Start the timer simultaneously, wait 2 minutes and register the first absorbance (A1).

5. After 4 minutes register the second absorbance (A2).

The procedure suggested above is suitable for photometer whose minimum solution volume necessary for measurement is lesser than or equal to 0.8 mL. Check whether there is the need to adjust the reaction volume to the photometer being used.

Sample and reagent volumes can be changed proportionally without any performance loss, and the calculation procedure remains the same. If the volumes are reduced, pay attention to the minimum solution volume for photometric detection. Sample volumes lower than 0.01 are critical and should be used carefully, as they increase the measurement's imprecision.

Calculation

Manual system . Calculate the difference between absorbances 1 and 2 for each calibrator (Δ Abs):

Calibrators		Absorbance		Δ Abs
N	Concentration (mmol/L)	Abs 1	Abs 2	Abs 1 - Abs 2
1	3.0	2.022	1.641	0.381
2	7.2	1.829	1.267	0.562

Calculate the factor according to the equation below:
[Cal. 1] = Calibrator 1 concentration
[Cal. 2] = Calibrator 2 concentration

$$\text{Factor} = \frac{[\text{Cal. 2}] - [\text{Cal. 1}]}{\Delta \text{Abs Cal. 2} - \Delta \text{Abs Cal. 1}}$$

$$\text{Factor} = \frac{7.2 - 3.0}{0.562 - 0.381} = \frac{4.2}{0.181} = 23.2$$

Factor = 23.2

Next, calculate the intersection using the following equation:

Intersection = Factor x Δ Abs Cal. 1 - [Cal. 1] = 23.2 x 0.381 - 3.0 = 8.83 - 3.0 = 5.83

Intersection = 5.83

To obtain the sample potassium concentration, calculate the absorbance delta of the sample and use it on the following equation:

Sample Concentration (mmol/L) = Sample Δ Abs x factor - intersection

Example

Sample	Absorbances		Δ Abs
	Abs 1	Abs 2	Abs 1 - Abs 2
1	2.036	1.621	0.415

Sample Concentration (mmol/L) = Sample Δ Abs x factor - intersection
Sample Concentration (mmol/L) = 0.415 x 23.2 - 5.83
Sample Concentration (mmol/L) = 3.80

Labtest

Calibration . Ensure the calibrators are at room temperature prior to being used. Homogenize the calibrators gently before use.

Manual and semi-automated systems
2-Point calibration
Points 1 and 2: Calibrators 1 and 2

Automated systems
Register daily the reagent blank using deionized water.

3-Point Calibration.
Point 0: deionized water.
Points 1 and 2: Calibrators 1 and 2.

Calibration frequency
when the internal quality control indicates so;
when using a new reagent lot;
when using new bottle of reagent from the same lot if a new calibration has been performed for the prior reagent bottle.

Parameters for automated analyzers

Parameter	Application
Reaction Type	Kinetic
Reaction Direction	Decreasing
Primary Wavelength	380 nm
Secondary Wavelength	700 nm
Temperature	37 ºC
Calibration	3 Points Point 0: Deionized water Point 1: Calibrator 1 Point 2: Calibrator 2
Calibration Model	Linear
Sample Volume*	5 µL
R1 Volume*	200 µL
Incubation	300 seconds at 37 ºC for R1 + sample
R2 Volume	50 µL
Reading 1 (Abs 1)	After 120 seconds of incubation of R1 + sample + R2 at 37 ºC
Reading 2 (Abs 2)	After 240 seconds of incubation of R1 + sample + R2 at 37 ºC

* The sample and reagent volumes can be modified proportionally without any loss in test performance, and the calculation procedure remains the same. In case of volume reduction it is crucial to observe the minimal necessary volume for photometric reading.

Operating interval . The measurement operating interval is 2.0 to 8.0 mmol/L.

Internal quality control . The laboratory must keep an internal quality control program with well-defined regulations, objectives, procedures, criteria of quality specifications and tolerance limits, corrective actions and registration of activities. Control materials should be used for measurement imprecision monitoring and determination of calibration deviation.
It is recommended to use the products Qualitrol - Labtest as internal quality control.

Expected values[2] . These values should be used only for orientation purposes. Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Serum: 3.5 - 5.1 mmol/L

Conversion: SI unit (mmol/L) x1 = Conventional unit (mEq/L)

Performance characteristics[3]

Recovery studies . A sample with potassium concentration equal to 5.6 mmol/L received different analyte amounts, and the following results were obtained:

Concentration (mmol/L)				Recovery rate
Initial	Added	Expected	Found	
5.60	1.20	6.80	6.82	100.3%
5.60	2.40	8.0	7.96	99.6%

The proportional systematic error estimated based upon decision level equal to 5.8 mmol/L is 0.003 mmol/L. The mean systematic error (0.05%) meets the optimal specification for total systematic error (≤±1.8%) based on the components of biological variation[4].

Method comparison . The proposed method was compared against Ion-Selective Electrode (ISE) method, and the following results were obtained:

	Comparative Method	Labtest Method
Samples	52	
Concentration Range (mmol/L)	2.7 - 7.7	2.5 - 7.8
Regression equation	Labtest method = 1.0703 x Comparative - 0.3042	
Correlation coefficient	0.990	

Using the regression equation, the systematic error (bias) was equal to 3.11% and 1.79% for samples with concentrations of 3.0 and 5.8 mmol/L, respectively

Imprecision . The imprecision studies were performed using two samples with concentrations of 4.62 mmol/L and 6.69 mmol/L.

Imprecision - Within Run

	N	Mean	SD	(%) CV
Sample 1	80	4.62	0.073	1.58
Sample 2	80	6.96	0.084	1.20

Labtest

Imprecision - Run-to-Run

	N	Mean	SD	(%) CV
Sample 1	80	4.62	0.081	1.77
Sample 2	80	6.96	0.122	1.77

The total error (random error + systematic error) estimated for the decision level of 5.8 mmol/L is equal to 4.71%. The results indicate that the method meets the desirable specification for Total Error (≤5.8%) based on the components of biological variation.

Analytical sensitivity . Detection limit: 0.219 mmol/L. The detection limit is equal to three standard deviations obtained with 80 measurements of a sample with potassium concentration equal to 4.62 mmol/L.

Effect of matrix dilution . A sample with potassium concentration equal to 8.0 mmol/L was used to evaluate the system response to matrix dilution using saline solution. Using dilution factors ranging from 1.6 to 2.1, the mean recovery found was 98%. The mean systematic error (2.0%) meets the minimum specification for total systematic error (≤±2.8%) based on the components of biological variation.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The water in the laboratory to prepare reagents and use in the measurements must have resistivity ≥1 megaohm, or conductivity ≤1 microsiemens and silicates concentration must be <0.1mg/L (Type II reagent water). The water for washing must be Type III, having resistivity ≥0.1 megaohms or conductivity ≤10 microsiemens. For the final washing, use Type II reagent water.

References

1. M.N. Berry, R. D. Mazzachi, M, Pejakolvc, and M. J. Peak Enzymatic Determination of Potassium Serum. Clin. Chem. 35/5, 817-820 (1989).

2. C.A. Burtis, E.R. Ashwood, D. E. Bruns - Tietz Text book of Clinical Chemistry and Molecular Diagnostics, 4 ed.

3. Labtest: Data on file.

4. Sociedad Española de Bioquímica Clínica y Patología Molecular. Available at: <http:// www.seqc.es/.../Base_de_datos_de_Variacion_biologica_%7C_Bases_de_datos_y_documentos_del_Comite_de_Garantia_de_la_Cali.> (accessed on 02/2011).

Presentation

Product	Reference	Content	
Enzymatic Potassium	125-1/33	R 1	1 X 24 mL
		R 2	1 X 9 mL
		CAL 1	1 X 1 mL
		CAL 2	1 X 1 mL
	125-1/41	R 1	1 X 31 mL
		R 2	1 X 10 mL
		CAL 1	1 X 1 mL
		CAL 2	1 X 1 mL
	125-2/41	R 1	2 X 31 mL
		R 2	2 X 10 mL
		CAL 1	1 X 1 mL
		CAL 2	1 X 1 mL

The number of tests in automated systems depends on the programmed parameters.

Consumer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label provided that the procedures and storage conditions indicated on the label and in this insert have been followed correctly.

Labtest Diagnóstica S.A.

CNPJ: 16.516.296 / 0001 - 38

Av. Paulo Ferreira da Costa, 600 - Vista Alegre - CEP 33400-000

Lagoa Santa . Minas Gerais Brasil - **www.labtest.com.br**

Consumer Service | e-mail: sac@labtest.com.br

Edition: November, 2011

Ref.: 120112

AUTOMATION PROTOCOL FOR LABMAX 240® - Enzymatic Potassium Ref. 125

Item Name	#	K125	1

DATA INFORMATION

UNITS	mmol/L
DECIMALS	1

ANALYSIS

TYPE	RATE
Main W.Length 1	380
Sub W.length 2	700
METHOD	Enzimático

CORR.

Y = (SLOPE) 1 X + (INTER) 0

CALIBRATION

TYPE	Linear		
STANDARD			
#1	@	#4	
#2	@	#5	
#3		#6	

NORMAL RANGE

	MALE		FEMALE	
	LOW	HIGH	LOW	HIGH
Serum				
Urine				
Plasma				
CSF				
Dialysis				
Other				

Positions 1 to 77 in the test panel.
@Insert the analyte concentration for Calibrator 1 (Ref. 125.3) and Calibrator 2 (Ref. 125.4)

Item Name	#	K125	2

ASPIRATION

KIND ○ Single ◉ Double

	VOLUME
SAMPLE	4.5
REAGENT1 VOL	175
REAGENT2 VOL	45

µL

Third Mix. ◉ OFF ○ ON

R1 Blank ◉ Water Blank ○ R1-Blank1

MONITOR

0 LEVEL POINT	1
SPAN	3

DATA PROCESS

READ

	START	END
MAIN	35	49
SUB		

FACTOR

Blank correction	1.000

ABSORBANCE LIMIT

LOW	-3.000
HIGH	3.000

ENDPOINT LIMIT	0
LINEAR CHECK(%)	99

PROZONE CHECK

	START	END	LIMIT(%)	
FIRST				
SECOND				◉ Low ○ High
THIRD				◉ Low ○ High

Item Name	#	K125	3

AUTO RERUN SW

○ ON ◉ OFF

AUTO RERUN RANGE (RESULTS)

	○ ON ◉ OFF Lower	○ ON ◉ OFF Higher
Serum		
Urine		
Plasma		
CSF		
Dialysis		
Other		

AUTO RERUN CONDITION (ABSORBANCE)

Absorbance Range

Lower ○ ON ◉ OFF

Higher ○ ON ◉ OFF

Prozone Range ○ ON ◉ OFF

Labtest

Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro
Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive

Ref.: 170309

Labtest