Universidade do Minho

Laboratórios Integrados I

Determinação do espectro de absorvência da reacção cinética para quantificação de creatinina no sangue, no plasma e na urina humanos

Engenharia Biomédica

2005/2006

Introdução

O sector da Saúde é hoje um dos mais dinâmicos e onde a capacidade de inovação é um imperativo estratégico e operacional. Neste trabalho pretende-se determinar quantitativamente a concentração de biomoléculas em fluidos biológicos, por espectrofotometria. A espectrofotometria (o estudo da interacção da radiação electromagnética com as biomoléculas) é, de entre as várias técnicas analíticas disponíveis em laboratórios de análises clínicas, a mais utilizada. A espectrofotometria pode ser utilizada para identificar uma biomolécula específica, determinar a sua estrutura, determinar a sua concentração e/ou quantidade (ex.: proteínas, aminoácidos) e determinar a actividade de uma enzima específica. O método a utilizar neste trabalho é um método espectrofotométrico e baseia-se na detecção colorimétrica por absorção óptica.

Objectivos

Actualmente, existem vários métodos espectrofotométricos para a quantificação de biomoléculas em fluídos, dos quais muitos estão a ser comercializados sob a forma de kits. O objectivo deste trabalho é determinar a curva de calibração de cada mistura para se obter uma relação entre a concentração da biomolécula a analisar e a intensidade da luz absorvida ou transmitida pela mistura. É necessário de igual modo, estudar a sensibilidade, a linearidade, a repetitividade e a reprodutibilidade do método. Os resultados obtidos permitirão calibrar o sistema de detecção do transdutor óptico.

Procedimentos

O método a utilizar é comercializado pela BioLabo Reagents^{TM} sob a forma de kit, o "*Creatinine kinetic method (Ref: 80107)*". É baseado no aumento de absorção a 490 nm que ocorre quando o complexo R1+R2 reage com a creatinina. O aumento na absorvância a 490 nm é directamente proporcional à concentração de creatinina nas amostras. O procedimento recomendado encontra-se em anexo. Este ensaio é realizado num volume de reagente de 1 ml e com cuvetes com um *lightpath* (caminho da luz) de 1 cm. Cada medição deve ser feita 3 vezes (3 misturas iguais) para testar a repetitividade do método.

Standard: 2 mg/dl.

Pretende-se construir a curva de calibração para as seguintes concentrações de creatinina: branco (reagente + H_2O), 2 mg/dl, 1.5 mg/dl, 1 mg/dl, 0.5 mg/dl, 0.25 mg/dl. Faça as diluições necessárias do standard para obter estas concentrações. Faça um volume de **500 µl** para cada concentração.

Utilize um volume total de reagente de **30 ml** (15 ml de R1 + 15 ml de R2).

Altere a razão reagente/standard para conseguir medir concentrações de 4 mg/dl, 8 mg/dl e 16 mg/dl. Verifique a linearidade do método.

Anexo

BIOLABO REAGENTS
w w w . b i o l a b o . f r

MANUFACTURER:
BIOLABO SA,
02160, Maizy, France

CREATININE
Kinetic method

Reagent for quantitative determination of creatinine in human serum, plasma or urines

REF 80107: (R1 : 1 x 125 ml, R2 : 1 x 125 ml, R3 : 1 x 10 ml)

TECHNICAL SUPPORT AND ORDERS
Tel : (33) 03 23 25 15 50
Fax : (33) 03 23 256 256

CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

Interconversion of phosphocreatine and creatine is a particular feature of the metabolism processes of muscle contraction. Creatine and phosphocreatine partially convert to a waste product, creatinine. Thus, the amount of creatinine produced each day is related to the muscle mass (and body weight), age, sex, diet or exercise and does not greatly vary from day to day. Because creatinine is endogenously produced and released into body fluids at a constant rate and its plasma levels are maintained within narrow limits, its clearance can be measured as an indicator of glomerular filtration rate (GFR).

PRINCIPLE (4)(5)

Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pretreatment step. This reaction has been improved (specificity, speed and adaptability) by the developement of an initial-rate method.

REAGENTS COMPOSITION

Vial R1 **BASE**

Xi : IRRITANT
R36/38 : Irritating to eyes and skin.
S26 : In case of contact with eyes, thouroughly wash with plenty of water and seek medical advice.

Disodium Phosphate	6.4	mmol/l
Sodium hydroxide	150	mmol/l

Vial R2 **DYE**

Sodium dodecyl sulfate	0.75	mmol/l
Picric acid	4.0	mmol/l
pH 4.0		

Vial R3 **STANDARD**

Creatinine 2 mg/dl (177 µmol/l)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal : Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Mix vial R1 and vial R2 contents (1 volume/1 volume). A graduated test-tube may be used.
Automated instrument : reagent R1 and R2 may be added separetely (see § **MANUAL PROCEDURE**).

STABILITY AND STORAGE

Store at 18-25°C, away from light.
- Unopened, reagents are stable until expiry date stated on the label.
- Once opened, reagents are stable at least for 6 months when free from contamination.
  Working reagent is stable at least for 30 days at 2-8°C.

Standard stability (vial R3) : Several weeks once opened (tranfer the requested quantity, recap and store at 18-25°C)

Discard any reagent if cloudy or if the absorbance of working reagent is > 0.300 at 490 nm.

This Kit can travel at room temperature.

SPECIMEN COLLECTION AND HANDLING (2)

Serum or heparinised plasma.

Urines : Collecte during precisely timed intervalles (4, 12 or 24 h). Dilute 1+19 in distilled or demineralised water before determination.
- Creatinine is stable in specimen :
  for 24 h at 2-8°C (freeze for longer storage).

INTERFERENCES (1) (2) (3) (5)

Hemolysis, bilirubin and lipemia may cause falsely negative results.

Bilirubin : See § **MANUAL PROCEDURE**, Procedure n°2.

Ascorbic acid, glucose and some antibiotics interfere also with the determination of creatinine according to Jaffe method.

Reading interval is the main determinant for the specificity of the Jaffe reaction ; some interferents act quickly (acetoacetate) and others slowly (proteins). The majority of kinetic methods recommends a reading interval beetwen 30 and 150 seconds.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
1. Normal and pathological control sera.

CALIBRATION

- Kit Standard (vial R3) or BIOLABO-Multicalibrator, REF 95015.
- Or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases :
1. When changing batch of reagent.
2. After maintenance operations on the instrument .
3. When control values obtained are out of range, even after using a new vial of fresh serum .

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QUALITY CONTROL

- BIOLABO EXATROL-N (normal values), REF 95010.
- BIOLABO EXATROL-P (pathological values), REF 95011.
- Assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases :

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions :

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

Serum or plasma		
Creatinine	mg/dl	[µmol / l]
Male	0.9 to 1.3	[80-115]
Female	0.6 to 1.1	[53-97]
Urines		
Creatinine	mg / kg / 24 h	[µmol / kg / 24 h l]
Male	14 to 26	[124-230]
Female	11 to 20	[97-177]

GFR (Glomerular filtration rate)	ml per minute
Adult < 40 years	120 (100 – 140)
Adult > 40 years	Physiologically decreased approx. 1% every year.

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES (PROCEDURE N°1)

Within run n = 20	*Normal level*	*High level*
Mean mg/dl	1.32	3.65
S.D. mg/dl	0.016	0.03
C.V. %	1.2	0.8

Between run n = 20	*Normal level*	*High level*
Mean mg/dl	1.09	4.63
S.D.mg/dl	0.065	0.125
C.V. %	5.9	2.7

Detection limit : approximately 0.2 mg/dl at 37°C.

Sensitivity for 1 mg/dl : approximately 18 mAbs/min at 37°C.

Comparison study with commercially available reagent:

y = 1.06 x – 0.051 r = 1.000

LINEARITY

The assay is linear up to 15 mg/dl (1327 µmol/l). Above, dilute the specimen with saline solution and reassay taking into account the dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at temperature of measurement.
Perform all the assays at constant temperature (see note 4).

Procedure n°1 : For non icteric specimen using "Working reagent"

Pipette in a 1 cm pathlength cuvette :	**Blank (optional)**	**Standard**	**Assay**
Working reagent	1 ml	1 ml	1 ml
Demineralised water	100 µl		
Standard		100 µl	
Specimen (Note 1)			100 µl
Mix well. After 30 secondes, record absorbance A1 at 490 nm (490-510) against reagent blank or distilled water. Exactly 2 minutes after the first reading, record absorbance A2.			

Procedure n°2 : For icteric specimen using "Bi-reagent"

Pipette in a 1 cm pathlength cuvette :	**Blank (optional)**	**Standard**	**Assay**
Reagent R1	0.5 ml	0.5 ml	0.5 ml
Demineralised water	100 µl		
Standard		100 µl	
Specimen (Note 1)			100 µl
Incubate for 5 minutes at constant temperature, then add:			
Reagent R2	0.5 ml	0.5 ml	0.5 ml
Mix well. After 30 secondes, record absorbance A1 at 490 nm (490-510) against reagent blank or distilled water. Exactly 2 minutes after the first reading, record absorbance A2.			

Notes :

1. Specimen : serum, plasma or diluted urines 1+19 in distilled water.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
3. Working reagent (see § **REAGENT PREPARATION**).
4. Perform this test at 37°C to optimise the sensitivity.

CALCULATION

Calculate the result as follows :

$$\text{Serum or plasma : Result} = \frac{\text{(A2 - A1) Assay}}{\text{(A2 - A1) Standard}} \times \text{Standard Concentration}$$

Urines diluted with 1+19 : Multiply the above result by dilution factor 20.

GFR (by creatinine clearance determination):

Using 24 h urine and serum creatinine

$$\text{Corrected Creatinine Clearance (ml/min)} = \frac{\text{UCr x V x 1.73}}{\text{SCr x BSA}}$$

UCr = Urine Creatinine in mg/dl or µmol/l
SCr = Serum Creatinine in mg/dl or µmol/l
V = Urine volume excreted in ml/min (24 h urine volume/1440)
BSA = Body Surface Area in m^2

OR

Using only serum creatinine (by Cockroft and Gault formula)

$$\text{Creatinine Clearance} = \frac{\text{140 – age in years x 2.12 x weight in Kg x K}}{\text{Serum Creatinine (µmol/l) x BSA (m}^2\text{)}}$$

K = 1.00 for men or K = 0.85 for women

REFERENCES

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(2) *Clinical Guide to Laboratory Test, 3rd Ed., N.W. TIETZ (1995) p. 186-188.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-190 to 3-211*
(4) *Fabiny D. L., et Ertingshausen G., Clin. Chem. (1971), 17, p.696-700.*
(5) *D. Labbé et al., Ann. Biol. Clin. (1996), 54, p. 285 – 298*

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Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number