Universidade do Minho

Laboratórios Integrados I

Determinação do espectro de absorvência da reacção enzimática para quantificação de glucose no soro, no plasma, na urina e no fluido cerebrospinal 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 "*Glucose GOD-PAP method (Ref: 80109)*". É baseado no aumento de absorção a 500 nm que ocorre quando o complexo vermelho *quinoneimine* reage com a glucose. O aumento na absorvância a 500 nm é directamente proporcional à concentração de glucose 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: 100 mg/dl.

Pretende-se construir a curva de calibração para as seguintes concentrações de cloreto: branco (reagente + H_2O), 100 mg/dl, 50 mg/dl, 25 mg/dl, 10 mg/dl, 5 mg/dl, 1 mg/dl. Faça as diluições necessárias do standard para obter estas concentrações. Faça um volume de **50 µl** para cada concentração.

Utilize um volume total de reagente de ≈**30 ml** (30 ml R1 e 450 µl de R2).

Altere a razão reagente/standard para conseguir medir concentrações de 200 mg/dl, 400 mg/dl e 600 mg/dl. Verifique a linearidade do método.

Anexo

BIOLABO REAGENTS
www.biolabo.fr

MANUFACTURER:
BIOLABO SA,
02160, Maizy, France

GLUCOSE
GOD-PAP

Reagent for quantitative determination of glucose
in human plasma, serum, cerebrospinal fluid (CSF) or urines

REF 80109	**R1**	1 x 250 mL	**R2**	1 x 3.75 mL	**R3**	1 x 5 mL
REF 80009	**R1**	1 x 500 mL	**R2**	1 x 7.5 mL	**R3**	1 x 5 mL
REF 87109	**R1**	6 x 250 mL	**R2**	6 x 3.75 mL	**R3**	1 x 5 mL
REF 16GL8	**R1**	6 x 1000 mL	**R2**	6 x 15 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) (6)

The glucose level in blood is maintained within a fairly narrow range under diverse conditions (feeding, fasting, or severe exercise) by regulatory hormones such as insulin, glucagon, or epinephrin. Measurement of glucose is one of the most frequently performed procedures in clinical chemistry laboratories in conjunction with other tolerance testing (Glucose tolerance test, Glucose 2h post-prandial…).

The most frequently encountered disorder of carbohydrate metabolism in blood is hyperglycemia due to diabetes mellitus.

Hyperglycemia higher than 300 mg/dL (16.5 mmol/L) may induce keto-acidosis and hyperosmolar coma.

In prolonged hypoglycemia, lower than 30 mg/dL (1.7 mmol/L), severe irreversible encephalic damage may occurs.

PRINCIPLE (4) (5)

Trinder Method. Glucose is oxidised by GOD to gluconic acid and hydrogen peroxide which in conjunction with POD, reacts with chloro-4-phenol and PAP to form a red quinoneimine. The absorbance of the coloured complexe, proportional to the concentration of glucose in the specimen is measured at 500 nm.

REAGENTS COMPOSITION

Vial R1 **ENZYMES-BUFFER**

Phosphate Buffer	150	mmol/L
Glucose oxidase (GOD)	≥ 20 000	UI/L
Peroxidase (POD)	≥ 1000	UI/L
4-Amino-antipyrine (PAP)	0.8	mmol/L

Vial R2 **CHROMOGEN**

Chloro-4-phenol	2	mmol/L

Vial R3 **STANDARD**

Glucose 100 mg/dL (5.55 mmol/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

Using a volumetric flask, measure the volume of demineralised water stated on the label of the vial R1(Enzymes-Buffer).

Transfer the contents of vial R1 into the flask and mix gently until complete dissolution (approximately 2 minutes). Then, add the contents of vial R2 and mix gently.

Vial R1 and R2 : If appropriate, use a non-sharp instrument to remove aluminium cap.

STABILITY AND STORAGE

Store at 2-8°C, away from light.

- Unopened : Reagents are stable until expiry date stated on the label.
- Once opened : Working reagent is stable for at least 1 year when free from contamination.
  Standard stability (vial R3) : Several weeks (transfer requested quantity, recap and store at 2-8°C).

Discard reagent if cloudy or if reagent blank at 500 nm is > 0.400.

This kit can travel for at least 1 week at room temperature.

SPECIMEN COLLECTION AND HANDLING (2)

Serum or plasma :

Separate promptly from cells to prevent glycolysis. If fluoride is used as a preservative, a decrease of 9 mg/dL (0.5 mmol/L) is seen within the first 2 hours, then concentration stabilises.

Glucose is stable in serum or heparinised plasma :

- for 8 h at 25°C
- for 72 h at 2-8°C

Glucose is stable in plasma (Sodium fluoride or iodoacetate) :

- for 24 h at room temperature.

CSF :

Process immediately to avoid falsely low results. Store at –20°C.

Urines :

Collect in dark bottle and store at 2-8°C. Preserve 24 h urines with 5 mL glacial acetic acid or 5 g sodium benzoate or sodium fluoride.

INTERFERENCES (3)

Ascorbic acid : No interference up to 10 mg/dL.
Total bilirubin : Negative interference above 20 mg/dL.
Direct bilirubin: No interference.
Hemolysis : No interference.
Lipemia : Positive interference above 626 mg/dL of triglycerides.

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.
2.Normal and pathological control sera.

Version AT 80009 25 02 2005

CALIBRATION

- Standard enclosed in the Kit (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. If control values are out of range, even after using a new vial of fresh control.

QUALITY CONTROL

- BIOLABO EXATROL-N (normal values) REF 95010.
- BIOLABO EXATROL-P (pathological values) REF 95011.
- Assayed control 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 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)

In serum or plasma :	**mg/dL**	**[mmol/L]**
Newborn. 1 day	40-60	[2.2-3.3]
Newborn. > 1 day	50-80	[2.8-4.4]
Children	60-100	[3.3-5.6]
Adult	74-106	[4.1-5.9]
60-90 years	82-115	[4.6-6.4]
> 90 years	75-121	[4.2-6.7]
In CSF :	**mg/dL**	**[mmol/L]**
Infant, Child	60-80	[3.3-4.4]
Adult	40-70	[2.2-3.9]

In 24 h urines : 1-15 mg/dL [0.1-0.8 mmol/L]

< 0.5 g/24 hours [<2.78 mmol/24 hours]

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

PERFORMANCES

Within run N = 30	***Normal level***	***High level***
Mean mg/dL	81	269
S.D. mg/dL	1.05	1.80
C.V. %	1.3	0.67

Between run N = 60	***Normal level***	***High level***
Mean mg/dL	81	284
S.D. mg/dL	0.97	3.01
C.V. %	1.2	1.06

Detection limit : approximately 10 mg/dL.

Sensitivity for 100 mg/dL: approximately 0.420 Abs at 500 nm.

Comparison with a commercially available reagent :

y = 0.969 x + 1.33 r = 0.9984

LINEARITY

The reaction is linear up to at least 500 mg/dL (28 mmol/L).

Above, dilute the specimen with saline solution and re-assay taking into account dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into well identified test tubes :	**Blank**	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL

Mix. Let stand for 10 minutes at 37°C or 20 minutes at room temperature.
Read absorbances at 500 nm (460-560) against reagent blank.
Coloration is stable for 15-20 minutes at 37°C, then slowly decreases.

Note : Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows :

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

REFERENCES

(1) *TIETZ Textbook of clinical chemistry. 3rd Ed. C.A. Burtis. E.R. Ashwood. W.B. Saunders (1999) p. 750-785.*
(2) *Clinical Guide to Laboratory Test. 3rd Ed.. N.W. TIETZ (1995) p. 268-277.*
(3) *YOUNG D.S.. Effect of Drugs on Clinical laboratory Tests. 4th Ed. (1995) p. 3-274 to 3-294.*
(4) *FARRANCE I. Clin. Biochem. reviews (1987). 8. p.55 to 68.*
(5) *TRINDER P.. Ann. Clin. Biochem.(1969). 6. p.24-27.*
(6) *BERNARD S., Biochimie clinique, 2cde éd.,Edition Maloine (1989), p.165-167.*

Made in France

Version : AT 80009 25 02 2005

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