Universidade do Minho

Laboratórios Integrados I

Determinação do espectro de absorvência da reacção para quantificação de cloretos no sangue, 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 "*Chloride method (Ref: 80005)*". É baseado no aumento de absorção a 500 nm que ocorre quando o complexo *Thiocyanate* reage com iões de cloreto. O aumento na absorvância a 500 nm é directamente proporcional à concentração de cloreto 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 mmol/L.

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

Altere a razão reagente/standard para conseguir medir concentrações de 200 mmol/L e 400 mmol/L. Verifique a linearidade do método.

Anexo

BIOLABO REAGENTS
www.biolabo.fr

MANUFACTURER:
BIOLABO SA,
02160, Maizy, France

CHLORIDE

Colorimetric method

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

REF 80005 R1 2 x 125 mL R2 1 x 5 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)

Chloride is the major extracellular anion. Together with sodium, chloride is significantly involved in maintenance of water distribution, osmotic pressure and anion-cation balance in extracellular fluids.
Decreased plasma Cl^- concentration is observed in salt-losing nephritis (e.g., chronic pyelonephritis) when hyponatremia is also observed. Hypochloremia is frequently observed in metabolic acidosis (e.g., diabetic acidosis and renal failure). Persistent gastric secretion and prolonged vomiting, whatever the causes, result in significant loss of Cl^- and ultimately in hypochloremia and depletion of total body Cl^-.
Increased plasma Cl^- concentration occurs with dehydratation, proximal renal tubular acidosis (RTA), acute renal failure, metabolic acidosis associated with prolonged diarrhea and loss of sodium bicarbonates…

PRINCIPLE (1) (4) (5)

$$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2SCN^-$$

$$3(SCN)^- + Fe^{3+} \longrightarrow Fe(SCN)_3$$

Chloride ions react with undissociated mercuric thiocyanate to form undissociated mercuric chloride and free thiocyanate ions. Thyocianate ions react with ferric ions to form a highly coloured reddish complex of ferric thiocyanate which absorbance, proportional to the amount of chloride in the specimen, is measured at 500 nm (450-500).

REAGENTS

Vial R1 **THIOCYANATE REAGENT**

Ferric nitrate	22.2	mmol/L
Chloride mercuric	0.55	mmol/L
Mercuric Thiocyanate	1.33	mmol/L
Nitric acid	30	mmol/L
Surfactant	1	mL/L

Vial R2 **STANDARD**

Chloride 100 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 and 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

Reagents are ready for use.

STABILITY AND STORAGE

Store at 18-25°C and away from light.

- Unopened : Reagents are stable upon expiry date stated on the label.
- Once opened : Reagent (vial R1) is stable for at least 3 months when free from contamination.
  Standard stability (vial R2) : Several weeks once opened (tranfer the requested quantity, recap and store at 18-25°C).

Discard any reagent if cloudy or if reagent blank at 500 nm > 0.100.

This Kit can travel at room temperature.

SPECIMEN COLLECTION AND HANDLING (2) (6)

Unhemolysed serum or heparinised plasma.

Urines or CSF.

Chloride is stable in the specimen for :
✓1 week at room temperature or 2-8°C.

INTERFERENCES (3)

Bilirubin :	No significant interference up to 100 µmol/L (6.0 mg/dl). Above, slightly over-estimated results which do not exceed 2 mmol/L beetwen 100 and 375 µmol/L of bilirubin.
Turbidity :	If opalescent, over-estimation approx. 4 to 8 mmol/L. If lactescent, results too over-estimated to be suitable. To reduce this interference, perform a specimen blank or bichromatic analysis (see § **MANUAL PROCEDURE**)
Haemoglobin :	Over-estimation of 5 mmol/L for an hemoglobin concentration in specimen of 205 µmol/L (330 mg/dl).
Ascorbic acid :	No significant interference up to 10 mg/dl of vitamin C in specimen.

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

MATERIALS REQUIRED BUT NOT PROVIDED

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

CALIBRATION

- Standard (vial R2) enclosed in the Kit 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 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.
- Other 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)

In serum or plasma

Chloride	mEq/L	[mmol/L]
In cord	96-104	[96-104]
Premature	95-110	[95-110]
0 to 30 days	98-113	[98-113]
up to 90 years	98-107(108)	[98-107(108)]
> 90 years	98-111	[98-111]

In 24 h Urines

Chloride	mEq/L	[mmol/L]
Newborn	2-10	[2-10]
Child < 6 years	15-40	[15-40]
6-10 years, M	36-110	[36-110]
6-10 years, F	18-74	[18-74]
10-14 years, M	64-176	[64-176]]
10-14 years, F	36-173	[36-173]
Adult	110-250	[110-250]
> 60 years	95-195	[95-195]

In CSF

Chloride	mEq/L	[mmol/L]
Child	110-130	[110-130]
Adult	118-132	[118-132]

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

PERFORMANCE CHARACTERISTICS

Within run n = 20	*Low level*	*High level*
Mean mmol/L	85.1	136.3
S.D. mmol/L	0.51	0.94
C.V. %	0.60	0.69

Between run n = 20	*Low level*	*High level*
Mean mmol/L	84.6	133.4
S.D. mmol/L	0.67	1.95
C.V. %	0.79	1.5

Detection limit : approximately 1.4 mmol/L

Sensitivity for 100 mmol/L : approximately 0.350 Abs at 500 nm.

Comparison study with a commercially available reagent:

y = 1.0391 x – 2.9153 r = 0.9944

LINEARITY

The reaction is linear beetwen 70 and 140 mmol/L. The maximum difference is ± 3% of the theoretical value. For values lower than 70 mEq/L, establish a calibration curve (with chloride solutions 20, 40, 60 mEq/L) or increase the specimen volume. Above 140 mmol/L, dilute specimen with chloride-free demineralised water and reassay taking into account dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimen 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 well. Let stand for 5 minutes at room temperature.
Record absorbances at 500 nm (450-500) against reagent blank.
Colour is stable for 30 minutes away from light.

Notes :

- ✓ Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
- ✓ Specimen blank may be performed replacing thiocyanate reagent by saline solution. This specimen blank is measured at 500 nm against saline solution and then deduced from the absorbance measured without specimen blank.
- ✓ A bichromatic analysis beetwen 500 and 600 nm allows to reduce of 50% the interference due to the turbidity, notwith-standing a loss of sensitivity of 10%.
- ✓ Sensitivity is better at 450-460 nm, but the reaction is more specific beetwen 480 and 500 nm.

CALCULATION

Calculate the result as follows :

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

REFERENCES

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Made in France

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