Universidade do Minho

Laboratórios Integrados I

Determinação do espectro de absorvência da reacção para quantificação de cálcio no soro, 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 "*Calcium Arsenazo III method (Ref: 90004)*". É baseado no aumento de absorção a 650 nm que ocorre quando o complexo *Arsenazo III* reage com o cálcio. O aumento na absorvância a 650 nm é directamente proporcional à concentração de cálcio 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: 10 mg/dl.

Pretende-se construir a curva de calibração para as seguintes concentrações de cloreto: branco (reagente + H_2O), 10 mg/dl, 7.5 mg/dl, 5 mg/dl, 2.5 mg/dl, 1 mg/dl, 0.5 mg/dl. Faça as diluições necessárias do standard para obter estas concentrações. Faça um volume de **100 µl** para cada concentração.

Altere a razão reagente/standard para conseguir medir concentrações de 20 mg/dl, 30 mg/dl e 40 mg/dl. Verifique a linearidade do método.

Anexo

BIOLABO REAGENTS
www.biolabo.fr

MANUFACTURER:
BIOLABO SA,
02160, Maizy, France

CALCIUM

Arsenazo III method

Reagent for quantitative determination of calcium in human serum and plasma, or urines.

REF 90004 R1 2 x 125 mL R2 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) (2)

Calcium fulfills a variety of roles in human physiology, not only as a structural factor in bones and teeth, but also in normal neuromuscular function and clotting of blood.
The level of serum calcium may be affected by intestinal malabsorption, by alterations in plasma proteins level, especially albumin, which should be measured concurrently with calcium.
Hypercalcemia is found in hyperparathyroïdism, multiple myeloma, bone and parathyroïdal neoplasms and in states with bones demineralisation.
Hypocalcemia is encountered in hypoparathyroïdism and in several cases of necrosis and acute pancreatitis.

PRINCIPLE (4)

At midly acidic pH, metallo-chromogen Arsenazo III combines with calcium to form a coloured complex which absorbance measured at 650 nm (640-660) is proportional to the amount of calcium in the specimen.

REAGENTS

Vial R1 **ARSENAZO III REAGENT**

Imidazol buffer pH 6.8 at 25°C	> 90	mmol/L
Arsenazo III	> 0.18	mmol/L
Surfactant	0.1	%
Preservative		

Vial R2 **STANDARD**

Calcium 10 mg/dL (2.5 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.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- 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.

REAGENT PREPARATION

Reagents are ready for use.

STABILITY AND STORAGE

Store at 18-25°C, away from light.

- Unopened :
  Reagents are stable until expiry date stated on the label.
- Once opened :
  Reagent R1 is stable at least for 3 months when free from contamination.

Standard stability (vial R2) : Several weeks once opened (tranfer requested quantity, recap and store at 18-25°C).

Discard any reagent if cloudy or if reagent blank at 650 nm > 0.400.

This kit can travel at room temperature.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Serum or heparinised plasma :
Do not use citrate, oxalate or EDTA. Blood obtained on fasting patient with minimal veinous occlusion and without exercise or after restoring circulation at least for 1 minute.

24 h Urines :
Acidify after collection with 20 to 30 mL HCl 6 N to dissolve calcium salts.
Dilute (1 + 2) with distilled water before performing the test.

Total calcium is stable in serum for :

- at least 7 days at 2-8°C.
- 6 months at -20°C.

Long-term freezing may lead to associated evaporation, lyophilisation or coprecipitation with fibrin (i.e. heparinised plasma) or lipids.

INTERFERENCES (3)

Hemolysis, icterus, lipemia, paraproteins and magnesium : Perform bichromatic or multi-wavelength analysis or specimen blank to reduce positive or negative interferences.

Handle with care specimens, calibrators and controls to avoid contamination by environnemental calcium. Use disposable tubes and cuvettes and clean glassware with HCl 1N, well rinse with demineralised water.

Plastic and glass containers may adsorbe calcium during storage, especially with diluted solution.

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

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MATERIAL REQUIRED BUT NOT PROVIDED

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

CALIBRATION

- Kit Standard (vial R2) 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 serum.

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)

TOTAL CALCIUM in serum :

Population	mg/dL	mmol/L
Premature	6.2-11.0	[1.55-2.75]
0-10 days	7.6-10.4	[1.90-2.60]
10 days –24 months	9.0-11.0	[2.25-2.75]
24 months –12 years	8.8-10.8	[2.20-2.70]
12 years -18 years	8.4-10.2	[2.10-2.55]
18-60 years	8.6-10.0	[2.15-2.50]
60-90 years	8.8-10.2	[2.20-2.55]
> 90 years	8.2-9.6	[2.05-2.40]

TOTAL CALCIUM in 24 h Urines : < 300 mg/24 h (< 7.5 mmol/24 h).

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

PERFORMANCES CHARACTERISTICS

Within run n = 20	*Normal Level*	*High Level*
Mean mg/dL	8.82	14.02
S.D. mg/dL	0.04	0.06
C.V. %	0.5	0.4

Between run n = 20	*Normal Level :*	*High Level*
Mean mg/dL	8.96	14.14
S.D. mg/dL	0.11	0.20
C.V. %	1.26	1.42

Detection limit : approximately 0.21 mg/dL

Sensitivity for 1 mg/dL : 0.070 Abs. at 650 nm.

Comparison with commercially available reagent :

y = 1.0259 x -0.1811 r = 0.9943

LINEARITY

The assay is linear up to 15.0 mg/dL (3.75 mmol/L). Above, dilute the specimen with demineralised water and reassay taking into account the dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Temperature should be held constant as the absorbance of the dye is temperature sensitive.

Pipette into well identified test tubes :	**Blank**	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
Distilled water	20 µL		
Standard		20 µL	
Specimen			20 µL
Mix well. Let stand for 1 minute at room temperature. Read absorbances at 650 nm (640-660) against reagent blank. The coloration is stable for 1 hour away from light			

Notes :

✓Bichromatic analysis : the 2nd wavelength is 700 nm.

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

CALCULATION

Calculate the result as follows :

Serum or plasma : $\text{Result} = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \times \text{Standard concentration}$

Urines diluted (1 + 2) : multiply the above result by dilution factor 3.

REFERENCES

(1) TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1395-1406, p.1435-1439.

(2) Clinical Guide to Laboratory Test, 3rd Ed., N.W. TIETZ (1995) p. 102-104.

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-115 à 3-125

(4) BAUER J. P., Affinity and stochiometry of calcium binding Arsenazo III, Anal. Biol. Chem.(1981), 110, p.61-72

Made in France

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