COD 12526 50 mL
STORE AT 2-8ºC
Reagents for measurement of zinc concentration Only for *in vitro* use in the clinical laboratory

ZINC

Automated Systems

BioSystems REAGENTS & INSTRUMENTS

CE

ZINC
BROMO-PAPS

PRINCIPLE OF THE METHOD

Zinc in the sample reacts with 5-Br-PAPS in alkaline medium forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS AND COMPOSITION

A1. Reagent. 2 x 15 mL. Sodium carbonate 240 mmol/L, pH 10.0.
A2. Reagent. 2 x 5 mL. Sodium citrate 170 mmol/L, Salicylaldoxime 24 mmol/L, preservatives.
B. Reagent. 1 x 10 mL. Sodium carbonate 50 mmol/L, 5-Br-PAPS 0.25 mmol/L.
S. Zinc standard. 1 x 3 mL. Zinc 2000 µg/dL equivalent to 10000 µg/dL (1529 µmol/L) of zinc according to the dilution factor of the sample. Aqueous primary standard.
WARNING: H317: May cause an allergic skin reaction. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.
Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank higher than 0.300 at 560 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

AUXILIARY REAGENTS

Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044).

REAGENT PREPARATION (Note 1)

Reagent A: pour the contents of the Reagent A2 vial into the Reagent A1 bottle. Mix gently. Other volumes can be prepared in the proportion: 3 mL Reagent A1 + 1 mL Reagent A2. Stable for 2 months at 2-8ºC.

Reagent B and Standard (S): Ready to use.

Reagents open and kept in the refrigerated compartment of the analyzer are stable 2 months.

ADDITIONAL EQUIPMENT

Analyzer, spectrophotometer or photometer able to read at 560 nm.

SAMPLES

Seminal plasma: Liquefy the fresh semen sample by incubation at 37ºC for 30 minutes. Centrifuge, in order to separate the spermatozoa, and decant the seminal plasma[2]. Stable at -20ºC.

Serum or heparinized plasma collected by standard methods. Stable 1 year at -20ºC, 2 weeks at 4-8ºC or 1 week at 20-25ºC.

CALIBRATION

It is recommended to use Zinc standard (S) for seminal plasma samples and the Biochemistry Calibrator for serum or heparinized plasma samples.
It is recommended to do a reagent blank every day and a calibration at least every 2 months, after reagent lot change or as required by quality control procedures.

PROCEDURE

Sample dilution (seminal plasma) (Note 1)

The standard, serum and heparinized plasma samples do not require pre-treatment.

1. Pipette into a test tube:

Seminal plasma	200 µL
Distilled water	800 µL

2. Mix gently. The diluted sample is stable 8 hours at 15-25ºC, 24 hours at 2-8ºC.

Automated procedure (Note 2)

		A25	A15
GENERAL	Test name	ZINC	ZINC
	Analysis mode	Differential bir	Differential bir
	Sample type	SEM/SER	SEM/SER
	Units	µg/dL	µg/dL
	Reaction type	increasing	increasing
	Decimals	0/1	0/1
	No. of replicates	1	1
	Test name in patient report	-	-
PROCEDURE	Reading	Monochromatic	Monochromatic
Volumes	Sample	3/15	3/15
	Reagent 1	240	240
	Reagent 2	60	60
	Washing	1,2	1,2
	Predilution factor	-	-
	Postdilution factor	2	2
Filters	Main	560	560
	Reference	-	-
Times	Reading 1	105 s	120 s
	Reading 2	420 s	432 s
	Reagent 2	120 s	144 s
CALIBRATION	Calibration type	specific/multiple	specific/multiple
	Calibrator replicates	3	3
	Blank replicates	3	3
	Calibration curve	-	-
OPTIONS	Blank absorbance limit	0.3	0.3
	Kinetic blank limit	-	-
	Linearity limit	30000/1250	30000/1250

Manual procedure (Notes 1, 3)

1. Pipette into test tubes:

	Reagent Blank	Sample Blank	Calibrator	Sample
Distilled water (SEM/SER)	12/60 µL	-	-	-
Zinc Standard (S)	-	-	12 µL	-
Biochemistry Calibrator	-	60 µL	60 µL	-
Seminal plasma	-	-	-	12 µL
Serum/Plasma	-	60 µL	-	60 µL
Reagent A	960 µL	1200 µL	960 µL	960 µL
Reagent B	240 µL	-	240 µL	240 µL

2. Mix thoroughly and incubate for 5 minutes at room temperature.
3. Measure the absorbance (A) of the Sample Blank at 560 nm against distilled water.
4. Measure the absorbance (A) of the Sample and Calibrator at 560 nm against Reagent Blank.
5. Calculate the zinc concentration using the following formula:

$$\frac{A_{Sample} - A_{Blank\ Sample}}{A_{Calibrator} - A_{Blank\ Calibrator}}$$

Seminal plasma	Serum / Plasma
x 10000 = µg/dL Zinc x 1529 = µmol/L Zinc	x C Calibrator = µg/dL zinc x C Calibrator = µmol/L zinc

REFERENCE VALUES

Seminal plasma[3,4]: 5000-50000 µg/dL (764-7644 µmol/L)
Serum[5]: 80-120 µg/dL (12-18 µmol/L).
These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Fertility Biochemistry Control (Cod. 18053), the Biochemistry Control Serum level I (Cod. 18005 and 18009) and level II (Cod. 18007 and 18010) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

The following data were obtained using an A25 analyser. Results are similar with A15. Details on evaluation data are available on request.

- Detection limit: 176 µg/dL=27 µmol/L (seminal plasma); 7.7 µg/dL=1.2 µmol/L (serum).
- Linearity limit: 30000 µg/dL=4586 µmol/L (seminal plasma); 1250 µg/dL=191 µmol/L (serum). For higher values dilute pretreated sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Seminal plasma	CV	n	Serum	CV	n
6990 µg/dL = 1070 µmol/L	1.6 %	20	69.8 µg/dL = 10.7 µmol/L	4.1 %	20
13600 µg/dL = 2080 µmol/L	0.9 %	20	95.7 µg/dL = 14.6 µmol/L	2.9 %	20

- Reproducibility (run to run):

Seminal Plasma	CV	n	Suero	CV	n
6990 µg/dL = 1070 µmol/L	2.1 %	25	69.8 µg/dL = 10.7 µmol/L	4.8 %	25
13600 µg/dL = 2080 µmol/L	2.0 %	25	95.7 µg/dL = 14.6 µmol/L	3.6 %	25

- Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.
- Interferences: Bilirubin (20 mg/dL) do not interfere. Lipemia (triglycerides 10 g/L) and hemolysis (hemoglobine 1.25 g/L) interfere. Other drugs and substances may interfere[6].

DIAGNOSTIC CHARACTERISTICS

Zinc is a transition metal essential in human nutrition. The most available dietary sources of zinc are read meat and fish. More than 200 zinc metalloenzymes occur in all six categories of enzyme systems.
Zinc levels in seminal plasma are associated with fertility. Its concentration is used in determining the secretory function of the prostate gland. Low values indicate an abnormal disruption of the secretory function, possibly as a result of obstruction of the conducts due to inflammation of acute or chronic nature[4].
Severe deficiencies in blood are related with depressed growth and stunting and increase incidence of infectious disease. Less severe deficiencies of zinc are related with immune function, diarrhea, altered cognitions, alopecia, eyesight defects and other adverse clinical outcomes. Ingestion of a zinc-contaminated diet involves high concentrations on blood and is associated with abdominal pain, diarrhea, nausea and vomiting. Heparinized plasma samples are preferred to serum for zinc analysis to avoid possible zinc contamination form erythrocytes, platelets and leukocytes during clotting and centrifugation[5].

NOTES

1. Contamination of glassware with zinc will affect the test. Use single use plastic material or acid-washed glassware.
2. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
3. Blank sample must be done in serum and heparinized plasma.

BIBLIOGRAPHY

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