Zinc

***In vitro* colorimetric method**

For the quantitative determination of zinc (60 Tests)

CE

439-14906-01
0307 D5

Intended use

Wako Zinc test is an *in vitro* diagnostic assay for the quantitative determination of zinc in serum, plasma, cerebrospinal fluid and urine.

Summary and explanation of test

Wako Zinc test is a kit of reagents that includes the sodium salt of 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol, (5-Br-PAPS) as the chromogenic component, and is used in a sensitive, colorimetric assay for zinc. Wako Zinc test is employed to quantitate accurately the amount of zinc in a specimen such as serum, plasma, cerebrospinal fluid and urine.

Principle of the method

A solution of trichloroacetic acid is added to the specimen to precipitate proteins, leaving zinc in solution. After proteins are removed, Color Reagents A and B are added to an aliquot of the supernatant fluid. Zinc binds to 5-Br-PAPS, forming a reddish-violet chelate. The absorbance of this reddish-violet chelate is measured at 560 nm, and is directly proportional to the amount of zinc in the specimen. Although the color reagents employed can also be used for the determination of iron, cop-per, cobalt, and nickel, reaction with these other metals is suppressed by masking agents that are contained in the assay kit. 5-Br-PAPS chelates zinc with a 2:1 molar stoichiometry.

Reagents

Contents and storage conditions

60 test unit containing:

R1:	2 Bottles	Color Reagent A	60 mL each
		0.2 M Carbonate- Bicarbonate buffer	pH 9.8
		5-Br-PAPS	0.07 mM
		Sodium Citrate	170 mM
		Dimethylglyoxime	4 mM
R2:	1 Bottle	Color Reagent B	30 mL
		Salicylaldoxime	29 mM
R3:	1 Bottle	Deproteinizing Reagent	30 mL
		Trichloroacetic Acid	7%
CAL:	1 Bottle	Standard Solution	10 mL
		as Zinc	200 µg/dL

Store all reagents at 2 - 25 °C under protection from light.

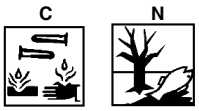
Preparation of the working solution

Mix R1 and R2 in a ratio of 4 : 1 (e.g., 20 mL of R1 is mixed with 5 mL of R2). After mixing the Working Solution can be used for up to two days at room temperature (below 35 °C), or for one week at 2 - 10 °C. After preparation, the blank test value increases slightly with time, but that has no effect on measured value.

Warnings and precautions

- For *in vitro* diagnostics use.
- For professional use.
- Not to be used internally in humans or animals.
- Do not use reagents past the expiration date stated on each reagent container label.
- Do not use the reagents described above for any purpose other than described herein.
- Do not mix or use the reagents from one test unit with those of another test unit which has a different lot number.
- The reagent is designed to be used manually or on commercial available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications.
- After opening the reagent, it is not recommended to store it for a long period of time. When the opened reagent is stored, cap the bottle and keep it at the specified temperature.
- The use of this assay and interpretation of its results is reserved to personnel adequately trained for performing and evaluating medical analytical and diagnostic procedures. Please refer to the respective local and national regulations.
- When discarding the reagents, dispose of them according to local or national regulations.
- This kit contains components classified as follows according to the European Directive 91/155/ EEC.

Hazard-determining components: Trichloroacetic Acid



C: corrosive
N: dangerous for the environment

Information pertaining to particular dangers for man and environment

Risk phrases:

34 Causes burns.

51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases:

26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

60 This material and its container must be disposed of as hazardous waste.

61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Physical or chemical indications of instability

The presence of precipitates in the reagents, or values of control sera outside of expected limits, may be an indication of reagent instability.

Specimen collection and preparation

Serum, plasma, cerebrospinal fluid and urine can all be analyzed by this procedure.

1. Take care to avoid contamination with chelators, such as EDTA, which cause artifactually low zinc concentration values to be measured. Heparin, citrate and oxalate used as anticoagulants don't affect the measurement.
2. In human blood, 75-85% of zinc exists in erythrocytes. 12-22% in plasma, and 3% in leukocytes. If hemolysis occurs, the amount of zinc that is measured in serum will be artifactually high. In such cases, another sample of blood should be collected. Zinc levels canbe determined not only in the serum, but also in plasma, CSF, and urine using a similar procedure.
3. Butyl rubber, which is often used as stopper of control serum bottle, contains somezinc. Dissolve the contents with „parafilm" in stead of butyl rubber as a stopper.
4. When sample of blood is collected, take care to avoid using apparatus* composed of rubber which contains zinc.

* ex. blood collecting tube, syringe.

Washing apparatus

Tap water may contain small amounts of zinc and larger amounts of iron or copper. The use of such tap water introduces positive errors into the determination of zinc in a specimen. All glassware, therefore, must be soaked in dilute hydrochloric acid or dilute nitric acid (hydrochloric acid or nitric acid diluted with 9 volumes of deionized water), wash briefly with tap water, then rinsed with deionized or distilled water. Glassware should then be dried well before use. If the variability between duplicate reagent blank measurements exceeds 3% contamination of the glassware should be suspected.

Assay procedure (manual)

All equipment must be free from zinc contamination. Wash Sample and Reagent containers with 1 +10 Triton x 100+Water, immersed in dilute hydrochloric or nitric acid for 1 to 2 hours and rinse with distilled or deionised water. Invert and allow to drip dry.

Wavelength:	560 nm (Hg 546 nm)
Cuvettes:	1 cm light path
Incubation Temperature:	15° - 35°C

Measure the absorbance of Sample and Standard against the Reagent Blank. For each batch use a Reagent Blank and one or two Standards.

GB.DE.FR.ES

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Details of procedure

Pipette into vials	Macro			Semi-Micro		
	Sample	Std.	Blank	Sample	Std.	Blank
Sample or Control	0.5 mL			0.2 mL		
Standard		0.5 mL			0.2 mL	
Dist. or deionized water			0.5 mL			0.2 mL
Deproteinizing Reagent	0.5 mL	0.5 mL	0.5 mL	0.2 mL	0.2 mL	0.2 mL
Mix well. Centrifuge for 10 minutes. at 3000r.p.m.						
Supernatant	0.5 mL	0.5 mL	0.5 mL	0.2 mL	0.2 mL	0.2 mL
Working Solution	2.5 mL	2.5 mL	2.5 mL	1.0 mL	1.0 mL	1.0 mL
Mix well. Incubate for 10 minutes. Measure absorbance of the Sample and standard against the Reagent blank. The final color is stable for 1 hour.						

Calculations

From the calibration curve

The zinc concentration that corresponds to the measured absorbance of the sample (A_S) is determined by interpolation on a calibration curve.

Calculation

$$\text{The zinc concentration in the sample (µg/dL)} = \frac{A_S}{A_{Std}} \times 200$$

Determination of Zinc in 24-hour Urine Specimens

Preparation of Specimen

Add one or two drops of concentrated hydrochloric acid to urine that was collected over 24 hours. Mix well to reduce the pH to between 3 and 4. After dissolving the sediment (to which zinc readily absorbs), the sample is ready for analysis.

Calculation for Urine

$$\text{Zinc in urine sample (µg/day)} = \frac{A_S}{A_{Std}} \times 200 \times 10 \times \text{urinary volume (L)}$$

Performance characteristics

Sensitivity: The theoretical sensitivity of this method expressed as absorptivity using a Hitachi 200 Spectrophotometer at 560 nm, is 1110 L/gm•cm for fresh reagent.

Accuracy: The accuracy of the Wako Zinc method was demonstrated by recovery studies in which known quantities of zinc were added to serum. Recovery: 96-101%

Precision: The precision of the Wako Zinc was established by analyzing a series of standards in replicate. CV% < 2%.

Specificity: The Specificity of the Wako Zinc method was determined by analysis of serum samples following the additions of potential interfering substances. See Table: Serum Zinc Additive Study

Table 1: Serum Zinc Additive Study

Additive				Serum with Additive
	Name	Final Concentration	Ordinary Amount	Zinc Concentration (µg/dL)
Bilirubin		None	-	71.3
		5mg/dL	-	72.5
		10	-	70.5
		15	-	71.9
		20	-	72.5
		25	-	
Hemoglobin		None	-	71.3
		200mg/dL	-	77.6
		400	-	85.8
		600	-	92.1
		800	-	97.2
		1000	-	104.1
Anticoagulants	None	-	-	71.3
	Citrate	2.0%	0.5%	72.5
	Heparin	0.1%	0.01%	71.3
	Oxalate	2.0%	0.2%	72.6
	EDTA	0.5%	0.1%	1.9
	NaF	3.0%	1.0%	73.8

Table 2: Serum Zinc Additive Study (Effect of Metal Ion)

Additive			Serum with Additive
Name	Final Concentration	Normal amount in Serum	Zinc Concentration µg/dL
None	-	-	72.9
Cu^{2+}	100 µg/dL	100 µg/dL	72.9
	300		74.1
	500		75.3
	1000		77.6
Fe^{2+}	100 µg/dL	120 µg/dL	73.5
	300		74.1
	500		76.4
	1000		80.0
Fe^{3+}	100µg/dL	120 µg/dL	73.1
	300		76.0
	500		77.6
	1000		81.7
Co^{2+}	500 µg/dL	trace amount	76.4
Ni^{2+}	500 µg/dL	trace amount	82.3
Ca^{2+}	50 mg/dL	10 mg/dL	72.9
Mg^{2+}	50 mg/dL	2 mg/dL	72.9
NH_4^+	2 mg/dL	60 µg/dL	71.7
PO_4^{3-}	25 mg/dL (as P)	4 mg/dL	73.5

Limitation of the procedure

When zinc value exceeds 500 µg/dL, dilute sample 1 + 1 with saline or distilled water, repeat assay and multiply the result by 2.

Expected values

Serum and Plasma

Men: 72.6-127.0 µg/dL (11.1-19.5 µ.mol/L)

Women: 70.0-114.0 µg/dL (10.7-17.5 µmol/L)

Low levels may be encountered in women during pregnancy or menstruation.

Children: 63.8-110.0 µg/dL (9.8-16.8 µmol/L)

New Born: 49.5-99.7 µg/dL (7.6-15.3 µmol/L)

Urine: 300-800 µg/24 h

Since expected values are affected by age, sex, diet, geographical location and other factors. Each laboratory should establish its own expected values for this procedure.

Quality control

A quality control program is recommended for all clinical laboratories.

References

1. Makino, T., Saito, D. Horiguchi, K. Kina: Clin. Chim. Acta 120:127 (1982). A highly sensitive colorimetric determination of serum zinc using water-soluble pyridylazo dye.
2. Oster, O., Huesgen, G., und Prellwitz, W. Präanalytische und analytische Probleme einer teilmechanisierten photometrischen Serumzinkbestimmung. Ärztl. Lab. 33, 177-185 (1987)
3. Johnsen, Ø. und Eliasson, R. Evaluation of a commercially available kit for the colorimetric determination of zinc in human seminal plasma. Int. J. Androl. 10, 435-440 (1987)
4. Kruse-Jarres, J.D. , Zinc (Zn) S. 347- 349 in Thomas, L., Hsg., Clinical Laboratory Diagnostics. Use and Assessment of Clinical Laboratory Results. Frankfurt/ Main, 1998.

Ordering information

Code No	Product	Package
439-14906	Zinc	60 Tests
410-00102	Wako Control Serum I	10 x 5 for mL
416-00202	Wako Control Serum II	10 x 5 for mL

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