

# Manual Procedure

Cat. No. 12650 R 1 x 50 ml

For 50 tests

## Zinc

Colorimetric test, 5-Br-PAPS method

### Liquid Reagent

#### Test principle

Zinc reacts with 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol (5-Br-PAPS) to form a red chelate complex in pH = 9.8. The increase of absorbance measure at 560 nm is proportional to the concentration of total zinc in the sample.

#### Concentrations in the test

Reagent R			
Bicarbonate buffer, pH = 9.8	500	mmol/L	
5-Br-PAPS	0.03	mmol/L	
Sodium citrate	68	mmol/L	
Dimethyl glyoxime	4	mmol/L	
Detergent			
<b>Standard :</b> The concentration as indicated on vial.			

#### Stability of reagent

**Reagent :** Liquid, ready for use.

Stability: Up to expiry date given on the label when stored at +2 → +8°C.

#### Specimen collection and handling

1. Non-hemolyzed serum and plasma.
2. Heparinized plasma could only be used, other anticoagulants should not be used. Free of hemolayz.
3. Blood specimen should be collected in trace element blood collection tube.
4. Remove serum from clot as soon as possible.
5. Stability in serum: 7 days at 2 – 8 °C.
6. 24/ hr. Urine: Collect in clean, plastic urine container with no metal cap or glued insert. Refrigerate after completion of collection.
7. Seminal fluid: Centrifuge the sample at 3000 rpm for 10-15 min. Stability of the sample 7 days at 2 – 8 °C. Dilute supernatant (1+99) with sodium chloride solution (0.9 %) and multiply the result by 100.

#### Standard

Zinc STD. Cat. No. 16221

#### Quality control

Meditrol N Cat. No. 15171

Meditrol P Cat. No. 15181

#### Procedure

Wavelength	Hg 546 nm 560 nm
Spectrophotometer	
Cuvette	1 cm light path
Temperature	20 – 25 °C
Measurement	Against reagent blank
Reaction	end point

#### Assay

	Blank	Standard	Sample
Double dist. water	50 µl	--	--
Standard	--	50 µl	--
Sample	--	--	50 µl
Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate for 8 min. at 20 – 25 °C. Read the absorbance (A). The final colour is stable for at least 30 min.			

#### Calculation

$$\text{Conc. Zinc } (\mu\text{g/dl}) = \frac{A_{\text{Sample}}}{A_{\text{STD.}}} \times \text{Conc. STD. } (\mu\text{g/dl})$$

$$\mu\text{g/dl} \xleftrightarrow[\times 6.537]{\times 0.153} \mu\text{mol/L}$$

#### Linearity

Up to 400 µg/dl (61.2 µmol/L).

If the result exceeds 400 µg/dl, repeat the test using diluted sample (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.

#### Precautions

1. Use only disposable plastic containers or iron free tubes and cuvettes. Avoid any contamination by the use of clean laboratory material.
2. Reagent contains sodium azide. Don't swallow. Avoid any contact with skin and mucous membranes. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

#### Reference range

Serum:	µg/dl	µmol/l
< 4 mth.	65 - 137	10 - 21
4 – 12 mth.	65 - 130	10 - 20
1 – 5 yr.	65 - 118	10 - 18
6 – 9 yr.	78 - 105	12 - 16
10 – 13 yr.	men	78 - 98
	women	78 - 118
14 – 19 yr.	men	65 - 118
	women	59 - 98
Adults	46 - 150	7 - 23

<b>Urine:</b>	( 300 – 800 ) mg/24hr.
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<b>Seminal fluid</b>	2 – 10 mg/dL 2000 – 10000 µg /dL
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#### Note:

Serum zinc levels are generally 5 -15 % higher than plasma levels due to zinc released from platelets and erythrocytes during clotting.

#### Reference

1. Johansen and R.Eliasson. Evaluation of a commercially available kit for colorimetric determination of zinc. International Journal of andrology, 1987, April 10 (2) : 435 - 440.
2. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.