ZINC

[Summary and Explanation]

Zinc is the second most abundant trace element in the body, found in more than 300 metalloenzymes. Carbonic anhydrase, alkaline phosphatase, DNA and RNA polymerases could be listed as important examples. Due to its multiple biochemical functions, zinc deficiency manifests by variety of conditions. Growth failure and stunning, alterations in immune function, diarrhea, skin lesions and alopecia are observed in cases of clinical deficiency. Subclinical deficiency has effects on immune function, synthesis and action of hormones, and neurological function.

[Principle of Assay]

Zinc found in the samples change the red-orange color of 5-Br-PAPS to light pink under alkaline conditions. The change of absorbance at 548 nm is proportional to total zinc level in the sample. The assay can be calibrated with zinc sulfate dissolved in deionized water.

[Reagent Composition]

Reagent 1: Buffer Solution
Surfactants

Reagent 2: Chromogen Solution
5-Br PAPS
Surfactants
Preservatives

[Intended Use]

This product can be used for quantitative *in vitro* determination of total zinc from human serum, plasma and urine.

[Stability and Preparation of Reagents]

R1 *Buffer*

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

R2 C*hromogen solution*

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

CAL and QC *Standard and levels*

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened. Any Zinc calibrator is applicable, however, Rel Assay calibrator and controls are recommended.

[Performance Characteristics]

Low linearity: 4 µg/dl - *High linearity:* 1000 µg/dl

If the results obtained are greater than linearity limit, dilute the sample 1/10 with deionized water and multiply the result by 10.

[Accuracy]

Results obtained with Rel Assay Zinc Measurement Kit and atomic absorption spectrometry, the gold standard method, are correlated.

The results obtained using 169 samples were the following:

Correlation coefficient (r): 0.98.

Regression equation: 0,97x + 2,41

The results of the performance characteristics depend on the analyzer used.

[Precision]

Intra-assay CV was determined with 30 replicates of 2 controls. The %CV result was 2.32 and inter-assay %CV result was 3.54.

[Normal range]

Adult Serum: 60-120 µg/dl

Newborn Serum: 50-100 µg/dl

Urine: 300-1200 µg/24h

Each laboratory is recommended to establish their own reference values.

Serum samples collected after at least 8 hours of fasting must be used for the test. Samples collected at a fed state causes decreased results.

Acute decrease of serum zinc concentrations could be observed during the post-operative 6 hours period.

[Handling method]

1. This product is designed to be used in the spectrophotometer, plate reader or automated biochemistry analyzer.

2. Could be used in various automated biochemistry analyzers, an example of operating application is shown below;

Sample	R1	R2	M.wav	S.wav
12.5µl	160µl	25µl	548 nm	None

3. R2 is added to the mixture two minutes after R1 and sample are mixed. Measurement is done at the end of five minutes.

Make sure that cuvettes of the auto-analyzer or plate reader are thoroughly cleaned before the assay to obtain accurate and sensitive results. Use Reagent Grade 1 water for the assay.

[Components]

All reagents are ready to use

	Content	Amount
R1	Buffer	60 ml
R2	Chromogen	10 ml
Standard	262 µg/dl Zn^{++}	3 ml
Level 1	33 µg/dl Zn^{++} (25-41)	3 ml
Level 2	327µg/dl Zn^{++} (261-393)	3 ml

[Storage and Validity Date]

Storage: This kit should be stored at 2-8°C.

Validity date: The validity of each reagent is indicated on the bottle and on the kit box.

(This kit is stable for up to expiry date when stored at 2-8°C after opened.)

[Sample]

Blood serum, plasma and urine could be used as sample.

Dilute semen samples with a ratio of 1/10 using 320 mOsm pH 7.4 phosphate buffer before evaluation.

Serum samples are stable up to 1 week stored at 4˚C, 2 months at -20˚C and 6 months at -80˚C.

Collect samples in collection tubes produced for trace element testing. Do not use tubes containing separation gel.

Serum samples collected after at least 8 hours of fasting must be used for the test.

[Procedure and Calculation]

Wavelength	548
Temperature	37 ^{0}C

Pipette into cuvette

	Samples	Standards (CAL, LEVELS, H_2O)
CAL, LEVELS, H_2O	-----	37.5
Sample	37.5	-----
Reagent 1	420	420
Reagent 2	75	75

Mix well

Mix, read the absorbance of sample, standards, control levels and water. Absorbance obtained from water is extracted from all samples, standard and control levels' absorbance. Extracted standard absorbance is accepted as 262 µg/dl. Calculate the factor and multiply the absorbance obtained from samples with factor to calculate the result. Level 1 is expected as 33±8 µg/dl and level 2 is expected as 327±66 µg/dl.

[Interferences]

No interferences were observed with bilirubin up to 18 mg/dL, hemoglobin up to 0.5 g/dL and triglycerides up to 1000 mg/dL.

EDTA interfere with the results.

Our studies have shown that there is no interference with other metal ions such as copper, iron, cobalt, magnesium and aluminum.

A list of drugs and other interfering substances with zinc determination has been reported by Young et. al (6).

In order to obtain best results, pipettes and cuvettes used for the assay must be cleaned thoroughly. Make sure that no wash solution is left on pipettes and in cuvettes.

[Recovery]

Serum pool was prepared and zinc concentration was measured. Then zinc concentrations were re-measured following addition of zinc sulfate in ratios of 1/10 and 1/100 into the same serum pool. As a result, recovery was found in between %95 and %105 range.

[Safety precautions and warning]

1. For *in vitro* diagnostic use only.
2. Do not pipette by mouth.
3. Exercise the normal precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.

References

1. Burtis CA, Burns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Seventh Ed. Elsevier Health Sciences; 2014. p. 493-496.

2. Rink L, Gabriel P. Zinc and the Immune System. Proc Nutr Soc. 2000;59:541-52

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5. Prasad AS. Zinc Deficiency. BMJ. 2003;326:409-10.

6. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.