REF ZR10400

Antigen
Σ 40

IVD CE

SemenZinc

(Seminal Zinc Test)

Application

The determination of the content of zinc in the liquid spermatozoa (ejaculate) is a biochemical marker of the prostate function. This test is used to determine the amount of zinc in seminal plasma and in serum, plasma, cerebrospinal fluid or urine.

Principle

Existing zinc binds to 5-Br-PAPS [2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol], and forms 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 sample.

Storage

15-25°C

Stability

At least 12 months from date of manufacture (unopened). After mixing the reagents 1 + 2 this working solution (AB) is durable at temperatures <35 C for two days or refrigerated (2-10°C) a week. Over time, the blank value increases slightly, but this has no effect on the calculated values.

Content

• Reagent 1 (5-Br-PAPS)	2 x 10 ml
• Reagent 2 (Salicylaldoxim)	5 ml
• Reagent 3 (TCA)	10 ml
• Reagent 4 (Zn-Standard)	3 ml
• 1 microtiter plate	

Required utensils and materials

- Distilled water
- Cover slides (18 x 18 mm)
- Ice
- Gloves
- Seminal plasma (100 µl)
- Slides
- Paper towels
- Microplate reader
- Pipettes and tips (10–100, 100–1000 µl)
- Tubes (1.5 or 2 ml)
- Tube holder
- Centrifuge
- Vortex mixer
- Trichloracetic acid
- 0.05% Triton X-100
- 0.01N HCl or HNO3

Important Note

All used tools must be free of zinc (pipette tips, distilled water, Trichloroacetic acid). For cleaning rinse tubes briefly with 0.05% Triton X-100 solution, put tubes 1-2 hours in 0.01N HCl or HNO_3 and rinse with distilled water. Leave to dry upside down. Prevent contamination with a separating agent (e.g. EDTA) to avoid falsely low zinc levels results. Avoid using tools that include zinc-containing rubber materials. Plasmas, as controls, used for determination of zinc, may no longer be sealed with rubber stoppers after reconstitution because of possible zinc contamination (use e.g. Parafilm). Anticoagulants such as heparin, citrate and oxalate do not interfere with measurement.

Quality Control of sample

In general, hemoglobin contains 75-85% of circulating zinc and other metal complexes. When contaminated with bloody ejaculate, there is danger of high zinc content due to hemolysis. First, microscope 5 µl of the ejaculate to exclude existing hemoglobin. If hemoglobin exists in the ejaculate (haematospermia), it is necessary to determine whether there has been already hemolysis. Brown dyed ejaculate is mostly hemolyzed and the specimen must be rejected. In light red samples there is intact hemoglobin and an additional cleaning step must be performed.

Preparation of work solution AB

For 1 microtiter plate mix 8 ml of reagent 1 and 2 ml of reagent 2 (ratio 4:1).

Preparation of zinc sulfate standard curve

Label 8 reagent tubes (Rx) and fill them:

		distilled H_2O	Reagent 4	Concentration of zinc
1. Rx	Blank (V_0)	150 µl	0 µl	0 µMol
2. Rx	Dilution V_1	135 µl	15 µl	10 µMol
3. Rx	Dilution V_2	120 µl	30 µl	20 µMol
4. Rx	Dilution V_3	105 µl	45 µl	30 µMol
5. Rx	Dilution V_4	90 µl	60 µl	40 µMol
6. Rx	Dilution V_5	60 µl	90 µl	60 µMol
7. Rx	Dilution V_6	30 µl	120 µl	80 µMol
8. Rx	Dilution V_7	0 µl	150 µl	100 µMol

It is recommended to re-create the standard curve in each experiment.

Procedure

Recovery of seminal plasma

from erythrocyte-free ejaculate:

Centrifuge 1 ml ejaculate 10 min at 1000x*g* (room temperature), transfer carefully the supernatant (seminal plasma) to a fresh tube and store on ice.

from erythrocytes containing ejaculate:

Centrifuge 1 ml ejaculate 10 min at 400x*g* (room temperature), transfer the supernatant (seminal plasma) carefully to a fresh tube and centrifuge again 10 min at 1000x*g*. Transfer the supernatant (seminal plasma) carefully to a new tube and store on ice.

1. Mix 100 µl seminal plasma with 100 µl of reagent 3.
2. Store on ice for 10 min.
3. Centrifuge 10 min at 1000x*g*.
4. Transfer carefully the supernatant to a new tube
5. Fill each well of microtiter plate with 50 µl according to the diagram below.
6. Add to all occupied wells 200 µl working solution (AB) and incubate 5 min at room temperature.
7. Measure at 560 nm the OD-values with a microplate reader. (OD=optical density, absorbance).

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	1	2	3	4	5	...	12
A	V_0	V_0	P_1	P_1			
B	V_1	V_1	P_2	P_2			
C	V_2	V_2	P_3	P_3			
D	V_3	V_3	…	…			
E	V_4	V_4	…	…			
F	V_5	V_5	…	…			
G	V_6	V_6	…	…			
H	V_7	V_7	P_n	P_n			

Standard dilution series: A1 and A2 to H1 and H2
Sample: A3 and A4 to C3 and C4, etc.

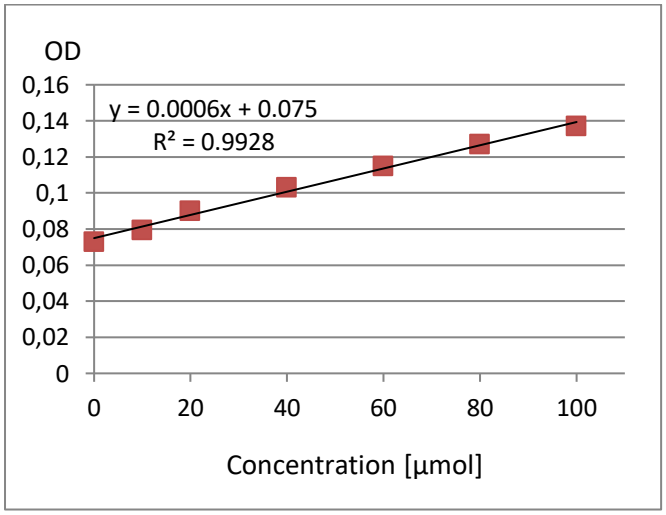
Ergebnis: Tabelle OD-Werte

	1	2	3	4	5	…	12
A	0.073	0.076	0.217*	0.209*			
B	0.079	0.08	0.342*	0.332*			
C	0.09	0.09	0.126	0.143			
D	0.107	0.104	0.094	0.099			
E	0.115	0.115	…	…			
F	0.127	0.127	…	…			
G	0,131	0,136	…	…			
H	0.1335	0.1335					

Standard straight line: A1; A2 to G1; G2 sample 1 to sample 4: A4 and A5 to D4 and D5

Evaluation objective: Determination of total zinc content in the ejaculate
The Zinc conzentration of the samples is to be converted to the total ejaculate. The lower reference range for zinc in semen is 2.4 µMol.

I. Creating of zinc sulfate standard curve:
The zinc sulfate standard curve is created using a spreadsheet program (e.g. Microsoft Excel®). On the x-axis are plotted the concentrations of zinc (micromoles) on the y-axis the OD values. This also allows the calculation of the coefficient of determination (R^2), which is used to determine the working precision (1 to 99% and from 0.01 to 0.99) by creating the standard curve. The value should be lower than 0.99, if not, the standard curve must be created again.



*optical density (OD) of samples, which are not within the range of the standard curve, cannot be used for concentration determination. These must repeated with adjusted sample volumes.

II. Calculating the concentration of the used sample:
The concentration of the sample can now be read or can be calculated by using the slope function. The general formula of the standard curve is:

$y = ax + c \Leftrightarrow x = (y - c) / a$

y = absorbance of the sample and of each used standard
x = concentration of used sample and standard (µMol)
a = slope function
c = linear constant of the curve

III. Calculation of the dilution factor:
Total volume (250 µl):
25 µl sample + 25 µl reagent 3 + 200 µl AB

Total volume / Sample volume = dilution factor
250 µl / 25 µl = **10**

IV. The total amount of zinc in ejaculate is to be calculated in three steps:

1. Calculation of the concentration x of the used sample, derived from the general formula of the absorbance.:

   x (mg / ml) = (y - c) / a

2. Calculation of the concentration of the undiluted

   x • Dilution factor

3. Determine the total amount of zinc sulphate, the result by the total volume (V) is multiplied in ml of seminal plasma

   x • dilution factor • V

Example

With 4 samples: Seminal plasma (P_1, 4 ml), seminal plasma (P_2, 3 ml), human serum (P_3, 20 ml), chicken serum (P_4, 30 ml)

Calculate the mean OD

	Standard OD	Sample OD	
V_0	0.073	0.213*	P_1
V_1	0.0795	0.337*	P_2
V_2	0.09	0.1355	P_3
V_3	0.103	0.0965	P_4
V_4	0.115	…	
V_5	0.127	…	
V_6	0.137	…	

*Absorbance value out of standard value

1. Obtain the concentration x of the used sample, derived from the absorbance:

   x (**µMol)** / ml = (y- c) / a

Measured zinc-concentration in diluted sample P_3
= (0.1355 – 0.075) / 0.0006 l / µmol
= **101 µMol / l**

2. Zinc-concentration in P_3 = 101 µMol / l • Factor
= 101 µMol / l • 10
= **1010 µMol / l**

3. Zinc-total in P_3
= 1010 µMol / l • 20 ml
= **20.2 µMol**

Example: OD below the highest standard value

	old	new
Volume sample	50 µl	25 µl
Volume AB	200 µl	225 µl

After measuring the result is to be multiplied by a factor of 20.

Example: OD below the lowest standard value

	old	new
Volume Sample	50 µl	100 µl
Volume AB	200 µl	150 µl

After measuring the result is to be multiplied by a factor of 5.

Safety information / Precautions

- All semen samples should be considered potentially infectious.
- Handle with all samples like HIV or hepatitis infected material.
- When working with samples and reagents wear always protective clothing (gloves, gowns, eye / face protection).
- Reagent 1 contains: sodium carbonate and sodium citrate. Both reagents cause irritation in the solid state; 5-Br-PAPS: not classified as dangerous; dimethylglyoxime: flammable in the solid state.
- Reagent 2 contains salicylaldoxime. Do not swallow, it is harmful and cause skin, eye and respiratory irritation.
- Reagent 3 contains trichloroacetic acid. It is corrosive and harmful to the environment
- Reagent 4 contains zinc sulfate. It is harmful and environmentally hazardous.

References

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