



# Ratios of Selenium/Trace Element Contents in Prostate Gland as Carcinoma's Markers



Vladimir Zaichick<sup>1\*</sup> and Sofia Zaichick<sup>2</sup>

<sup>1</sup>Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia

<sup>2</sup>Laboratory of Dr. Gabriela Caraveo Piso, Northwestern University, USA

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\*Corresponding author: Vladimir Zaichick, Radionuclide Diagnostics Department, Medical Radiological Research Centre, 4, Korolyev St., MRRC, Obninsk 249036, Russia, Fax: +7 (495) 956 1440; Tel: +7 (48439) 60289; Email: vezai@obninsk.com

## Abstract

The aim of the study was to evaluate whether significant changes in the prostatic tissue levels of ratios Se/trace element contents exist in the malignantly transformed prostate. Contents of Se and other 42 trace elements (Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr) in normal (N, n=37, age range 41-87 years), benign hypertrophic (BPH, n=32, age range 56-78), and cancerous human prostate (PCa, n=60, age range 40-70) were investigated.

Measurements of trace element contents were performed using a combination of instrumental neutron activation analysis and inductively coupled plasma mass spectrometry. Then the levels of ratios Se/trace element contents in every sample were calculated. It was observed that the ratio to Se of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Dy, Er, Fe, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Se/Ag, Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

**Keywords:** Trace elements; Trace element content ratios; Prostate; Benign prostatic hypertrophy; Prostatic carcinoma; Neutron activation analysis; Inductively coupled plasma mass spectrometry

**Abbreviations:** INAA-LLR: The Instrumental Neutron Activation Analysis with High Resolution Spectrometry Of Long-Lived Radionuclides; ICP-MS: The Inductively Coupled Plasma Mass Spectrometry; CRM: The Certified Reference Materials

## Introduction

The prostate gland may be a source of many health problems in men past middle age, the most common benign prostatic hyperplasia (BPH), and prostatic carcinoma (PCa). BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra and can significantly impair quality of life. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50 and greater than 90% of men over 70 years old [1,2]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males. Except for lung cancer, PCa is the leading cause of death from cancer [3-8]. Although the etiology of BPH and PCa is unknown, some trace elements have been highlighted in the literature in relation to the development of these prostate diseases [9-29].

Trace elements have essential physiological functions such as maintenance and regulation of cell function and signaling, gene regulation, activation or inhibition of enzymatic reactions, neurotransmission, and regulation of membrane function.

Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [30].

In earlier reported studies [31-69] significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed. In particular, it was shown that the average mass fraction of some trace elements in BPH were higher than normal levels, while those in adenocarcinoma were lower than in healthy prostatic parenchyma [60,61,66-68]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the ratios of chemical element contents changed in opposite directions during malignant transformation of prostate. For example, a significant informative value of Zn/Se content as a tumor marker

for PCa diagnostics was shown by us [70]. Hence it is possible that besides Zn, the ratio of Se to other trace elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Currently number of methods was applied for the measurement of chemical elements contents in samples of human tissue. Among these methods, the instrumental neutron activation analysis with high resolution spectrometry of long-lived radio nuclides (INAA-LLR) is a non-destructive and one of the most sensitive techniques. It allows measure the chemical element contents in few milligrams tissue without any treatment of sample. Nondestructive method of analysis avoids the possibility of changing the content of trace elements in the studied samples [71-74], which allowed for the first time to obtain reliable results. However, INAA-LLR allows only determine the mean mass fractions of 10-11 trace elements in the samples of normal and cancerous prostate glands [15,28]. The inductively coupled plasma mass spectrometry (ICP-MS) is more power analytical tool than INAA-LLR [18], but sample digestion is a critical step in elemental analysis by this method. In the present study two these analytical methods were used and the results, obtained for some trace elements by ICP-MS, were under the control of INAA-LLR data.

The present study had three aims. The main objective was to obtain reliable results about the 43 trace elements: Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr contents in intact prostate of healthy men aged over 40 years and also in the prostate gland of age-matched patients, who had either BPH or PCa, combining in consecutive order non-destructive INAA-LLR with destructive ICP-MS method. The second aim was to calculate Se/trace element content ratios for every sample and compare the levels of these ratios in normal, hyper plastic, and cancerous prostate. The third and final aim was to evaluate the ratios of Se/trace element contents for diagnosis of prostate cancer. All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

### Material and Methods

#### Samples

The patients studied (n=92) were hospitalized in the Urological Department of the Medical Radiological Research Centre (Obninsk, Russia). All of them were European-Caucasian, citizens of Moscow and Obninsk (a small city in a non-industrial region 105km south-west of Moscow). Trans rectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. The age of 32 patients with BPH ranged from 56 to 78 years, the mean being  $66\pm 6$  (M $\pm$ SD) years. The 60 patients aged 40-79 suffered from PCa (stage T1-T4).

Their mean age was  $65\pm 10$  (M $\pm$ SD) years.

Intact prostates (N) were removed at necropsy from 37 men aged 41-87 who had died suddenly. All deceased were European-Caucasian, citizens of Moscow. Their mean age was  $55\pm 11$  (M $\pm$ SD) years. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobes of their prostates, within 2 days of death and then the samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. A histological examination was used to control the age norm conformity, as well as to confirm the absence of micro adenomatosis and latent cancer [14,15,20,28].

#### Sample preparation

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The sample weighing about 10mg (for biopsy materials) and 50-100mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with double rectified alcohol before hand and placed in a nitric acid-washed quartz ampoule.

After INAA-LLR investigation, the prostate samples were taken out and used for ICP-MS method. The samples were decomposed in autoclaves; 1.5mL of concentrated HNO<sub>3</sub> (nitric acid at 65%, maximum (max) of 0.0000005%Hg; GR, ISO, Merck) and 0.3mL of H<sub>2</sub>O<sub>2</sub> (pure for analysis) were added to prostate tissue samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 3h at 160-200°C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20mL) and transferred to the plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO<sub>3</sub>+H<sub>2</sub>O<sub>2</sub>+ deionized water), and the resultant solutions were used as control samples.

#### Instrumentation and methods

A vertical channel of a nuclear reactor was applied to determine the trace element mass fractions by INAA-LLR. The quartz ampoule with prostate samples and certified reference materials was soldered, positioned in a transport aluminum container, and exposed to a 24 hour neutron irradiation in a vertical channel with a neutron flux of  $1.3\cdot 10^{13}$  n.cm<sup>-2</sup>.s<sup>-1</sup>. Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer used for INAA-LLR included the 100cm<sup>3</sup> Ge (Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9keV on the <sup>60</sup>Co 1332keV line. Other details of the INAA-LLR analysis were presented in our previous publication [15].

An ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA) was used to determine the content of trace elements by ICP-MS. The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. Information detailing with the ICP-MS method used was presented in our previous publication [18].

### Certified reference materials

For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal muscle and IAEA HH-1 Human hair from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRMs were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications [15,18].

### Results

**Table 1:** INAA-LLR data (M±SD) of chemical element mass fraction in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values (mg/kg, dry mass basis).

Element	IAEA H-4 Animal Muscle Certificate	This Work Results	IAEA HH-1 Human Hair Certificate	This Work Results
Ag	-	0.033±0.008	0.19 <sup>a</sup>	0.18±0.05
Au	0.07 <sup>a</sup>	<0.01	0.03 <sup>a</sup>	0.028±0.009
Br	4.1±1.1	5.0±0.9	4.2 <sup>a</sup>	3.9±1.6
Cd	0.04 <sup>a</sup>	<2	0.26 <sup>a</sup>	0.28±0.11
Ce	0.02 <sup>a</sup>	<0.1	-	≤0.4
Co	0.003 <sup>a</sup>	0.0034±0.0008	6.0±1.2	5.4±1.1
Cr	0.06 <sup>a</sup>	0.071±0.010	0.27 <sup>a</sup>	≤0.3
Cs	0.12 <sup>a</sup>	<0.05	-	≤0.01
Fe	49.1±6.5	47.0±1.0	23.7±9.8	25.1±4.3
Gd	-	<0.02	-	≤0.1
Hg	0.014 <sup>a</sup>	0.015±0.004	1.7±0.2	1.54±0.14
La	0.01 <sup>a</sup>	<0.5	0.01 <sup>a</sup>	≤0.2
Mn	0.52 <sup>a</sup>	0.55±0.11	0.85±0.25	0.93±0.16
Nd	-	<0.1	-	≤0.2
Rb	18.7±3.5	23.7±3.7	0.94 <sup>a</sup>	0.89±0.17
Sb	0.006 <sup>a</sup>	0.0061±0.0021	0.03 <sup>a</sup>	0.033±0.009
Sc	0.0006 <sup>a</sup>	0.0015±0.0009	-	≤0.01
Se	0.28±0.08	0.281±0.014	0.35±0.04	0.37±0.08
Sm	-	<0.01	-	≤0.01
Tb	-	<0.03	-	≤0.005
Th	-	<0.05	-	≤0.02
U	-	<0.07	-	≤0.006
Yb	-	<0.03	-	≤0.005
Zn	86.3±11.5	91±2	174±32	173±17
Zr	-	<0.3	-	<0.1

M: Arithmetical Mean; SD: Standard Deviation, a: Information Values

### Computer programs and statistic

A dedicated computer program for INAA mode optimization was used [75,76]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. For elements investigated by both INAA-LLR and ICP-MS methods the mean of all results was used. Using the Microsoft Office Excel software Se/trace element contents for each trace element in every sample were calculated. Then arithmetic mean±standard error of mean was calculated for trace element mass fraction and for ratios of Se/trace element mass fraction in normal, benign hyper plastic and cancerous prostate. The difference in the results between BPH and N, PCa and N, as well as PCa and BPH was evaluated by parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. Values of p<0.05 were considered to be statistically significant. For the construction of "individual data sets for Se/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate" diagrams the Microsoft Office Excel software was also used.

**Table 2:** ICP-MS data (M±SD) for chemical element contents in Certified Reference Materials (mg/kg, dry mass basis).

Element	Soya Bean Flour (INCT-SBF-4)		Tea Leaves (INCT-TL-1)		Mixed Polish Herbs INCT-MPH-2	
	Certificate	This work	Certificate	This work	Certificate	This work
Ag	-	0.0034±0.0008	-	≤0.0064	-	<0.001(DL)
Al	45.5±3.7	37.1±1.4	2290±280	2248±61	670±111	485±79
Au	-	<0.001(DL)	-	<0.001(DL)	-	<0.001(DL)
B	39.9±4.0	34.5±1.4	26 <sup>a</sup>	24.8±1.2	-	28.8±8.1
Be	-	0.0021±0.0019	-	0.020±0.004	-	0.021±0.002
Bi	-	<0.001(DL)	-	0.010±0.002	-	0.07±0.002
Br	2.40±0.17	4.70±0.64	12.3±1.0	6.8±1.6	7.71±0.61	<7.0(DL)
Cd	-	0.0208±0.0045	0.030±0.004	0.023±0.004	0.199±0.015	0.194±0.0035
Ce	-	0.0364±0.0057	0.79±0.08	0.74±0.07	1.12±0.10	1.12±0.20
Co	0.096±0.006	0.0908±0.0080	0.39±0.04	0.37±0.04	0.210±0.025	1.92±0.009
Cr	-	≤0.5	1.9±0.2	1.7±0.4	1.69±0.13	1.60±0.37
Cs	0.130±0.004	0.1253±0.0057	3.91±0.37	3.65±0.19	0.076±0.007	0.063±0.005
Dy	-	0.0014±0.0002	-	0.167±0.010	-	0.055±0.008
Er	-	0.0007±0.0001	-	0.098±0.006	-	0.027±0.003
Gd	-	0.0018±0.0004	-	0.190±0.010	-	0.076±0.018
Hg	-	<0.02(DL)	0.005±0.001	≤0.022	0.018±0.002	0.019±0.005
La	0.019±0.002	0.0144±0.0045	1.00±0.07	0.95±0.05	0.57±0.05	0.54±0.11
Li	-	0.0047±0.0018	-	0.217±0.034	-	0.574±0.044
Mn	32.3±1.1	30.0±1.0	1570±110	1628±145	191±12	197±5
Mo	5.99±0.35	5.66±0.28	-	0.052±0.009	0.52 <sup>a</sup>	0.53±0.01
Nb	-	0.0057±0.0023	-	0.044±0.031	-	0.032±0.001
Nd	-	0.0119±0.0036	0.81 <sup>a</sup>	0.81±0.06	0.46±0.09	0.47±0.10
Ni	3.12±0.18	2.57±0.20	6.1±0.5	5.3±0.7	1.57±0.16	1.62±0.10
Pb	-	0.068±0.023	1.8±0.2	1.5±0.3	2.16±0.23	2.07±0.32
Pr	-	0.0027±0.0007	-	0.200±0.013	-	0.124±0.027
Rb	31.7±1.7	30.8±2.8	81.5±6.5	80.9±6.7	10.7±0.07	10.9±0.4
Sb	-	0.0067±0.0044	0.050 <sup>a</sup>	0.032±0.011	0.065±0.009	0.053±0.014
Se	-	<0.1(DL)	0.076 <sup>a</sup>	≤0.12	-	0.088±0.026
Sm	-	0.0018±0.0006	0.18±0.02	0.17±0.01	0.094±0.008	0.087±0.021
Sn	-	<0.03(DL)	-	0.35±0.06	-	-
Tb	-	0.00023±0.00004	0.027±0.002	0.028±0.002	0.014±0.001	0.010±0.002
Th	0.007±0.001	0.0069±0.0030	0.034±0.005	0.029±0.011	0.154±0.013	0.136±0.022
Ti	-	0.93±0.15	30 <sup>a</sup>	32±6	34 <sup>a</sup>	20.7±4.9
Tl	-	0.0011±0.0002	0.063±0.005	0.065±0.003	0.029 <sup>a</sup>	0.032±0.002
Tm	-	0.00011±0.00002	0.017 <sup>a</sup>	0.015±0.001	-	0.0037±0.0003
U	-	0.0012±0.0007	-	0.009±0.001	0.049 <sup>a</sup>	0.038±0.011
Y	-	0.0069±0.0011	-	0.904±0.098	-	0.271±0.032
Yb	-	0.0004±0.0001	0.120±0.013	0.104±0.007	0.053±0.007	0.023±0.002
Zn	52.3±1.3	54.8±6.6	34.7±2.7	36.0±3.7	33.5±2.1	32.0±6.1
Zr	-	0.0295±0.0093	-	0.30±0.12	-	0.400±0.040

M: Arithmetical Mean; SD: Standard Deviation, a: Information Values.

**Table 3:** Mean values (M±SEM) of the trace element mass fraction (mg/kg, dry mass basis) in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

Element	Symbol	Prostatic Tissue		
		N 41-87 year (n=37)	BPH 56-78 year (n=32)	PCa 40-79 year (n=60)
Silver	Ag	0.038±0.006	0.0415±0.0090	0.252±0.030
Aluminum	Al	34.2±3.5	24.4±3.2	328±73
Gold	Au	0.0041±0.0008	0.00257±0.00077	0.0297±0.0056
Boron	B	1.04±0.18	1.51±0.26	12.6±3,7
Berillium	Be	0.00094±0.00007	0.000918±0.000042	0.0137±0.0022
Bismuth	Bi	0.029±0.011	0.140±0.042	1.75±0.27
Bromine	Br	27.9±2.9	30.6±3.4	99.9±8.9
Cadmium	Cd	1.12±0.13	1.07±0.43	0.425±0.099
Cerium	Ce	0.0309±0.0050	0.0128±0.0019	0.101±0.013
Cobalt	Co	0.0467±0.0064	0.0617±0.0084	0.0336±0.0040
Cromium	Cr	0.56±0.08	1.00±0.10	2.34±0.32
Cesium	Cs	0.0339±0.0033	0.0235±0.0025	0.0389±0.0039
Dysprosium	Dy	0.00293±0.00049	0.00156±0.00024	0.00771±0.00110
Erbium	Er	0.00148±0.00023	0.00072±0.00013	0.00297±0.00038
Iron	Fe	111±9	133±11	165±15
Gadolinium	Gd	0.00290±0.00041	0.00153±0.00027	0.00945±0.00173
Mercury	Hg	0.052±0.008	0.259±0.029	0.122±0.019
Holmium	Ho	0.00057±0.00008	0.00032±0.00005	0.00178±0.00022
Lanthanum	La	0.080±0.020	0.0385±0.0073	0.969±0.537
Lithium	Li	0.0419±0.0055	0.0385±0.0073	0.251±0.054
Manganese	Mn	1.34±0.08	1.19±0.09	6.99±1.35
Molybdenum	Mo	0.282±0.038	0.167±0.009	0.298±0.035
Niobium	Nb	0.0054±0.0012	0.0102±0.0079	0.0052±0.0002
Neodymium	Nd	0.0137±0.0021	0.0062±0.0009	0.0413±0.0065
Nickel	Ni	3.10±0.51	3.22±1.06	6.96±1.04
Lead	Pb	2.39±0.56	0.69±0.16	1.81±0.35
Praseodymium	Pr	0.00353±0.00053	0.00149±0.00027	0.00973±0.00174
Rubidium	Rb	13.3±0.9	14.3±0.8	8.71±0.66
Antimony	Sb	0.043±0.006	0.163±0.036	0.490±0.059
Scandium	Sc	0.0294±0.0053	0.0257±0.0040	0.0116±0.0015
Selenium	Se	0.75±0.05	1.11±0.07	0.56±0.08
Samarium	Sm	0.0027±0.0004	0.0014±0.0004	0.0095±0.0029
Tin	Sn	0.32±0.06	0.108±0.029	1.28±0.24
Terbium	Tb	0.00039±0.00006	0.00017±0.00003	0.00089±0.00012
Thorium	Th	0.0033±0.0007	0.0018±0.0003	0.0495±0.0123
Titanium*	Ti*	2.82±0.64	1.52±0.20	8.60±2.20
Thallium	Tl	0.0014±0.0001	0.00202±0.00057	0.0219±0.0056
Thulium	Tm	0.00024±0.00003	0.000151±0.000021	0.000535±0.000111
Uranium	U	0.0070±0.0021	0.0021±0.0009	0.0068±0.0013
Yttrium	Y	0.0187±0.0043	0.0071±0.0012	0.0340±0.0038
Ytterbium	Yb	0.00141±0.00025	0.00083±0.00020	0.00174±0.00039
Zinc	Zn	1031±129	1271±102	136±10
Zirconium	Zr	0.036±0.006	0.091±0.036	2.13±0.89

M: Arithmetic Mean, SEM: Standard Error of Mean, \*Titanium tools were used for sampling and sample preparation.

Table 1 & 2 depict our data for trace element mass fractions in CRMs measured using INAA-LLR and ICP-MS, respectively, as well as the certified values of these materials. Table 3 represents mean values±standard error of mean (M±SEM) of the Ag, Al, Au,

B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

**Table 4:** Mean values (M±SEM) of the Se mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

Ratio	Prostatic Tissue		
	N 41-87 year (n=37)	BPH 56-78 year (n=32)	PCa 40-79 year (n=60)
Se/Ag	35.2±5.7	57.0±12.2	2.55±0.51
Se/Al	0.0301±0.0032	0.0456±0.0051	0.00402±0.0018
Se/Au	362±63	536±94	62.9±26.2
Se/B	1.36±0.21	0.730±0.141	0.0686±0.0145
Se/Be	956±97	1042±102	55.9±11.7
Se/Bi	216±55	19.6±6.8	1.92±1.66
Se/Br	0.0369±0.0039	0.0372±0.0044	0.00748±0.00143
Se/Cd	0.990±0.160	1.92±0.48	2.01±0.21
Se/Ce	41.7±6.6	88.7±13.6	9.12±2.29
Se/Co	21.5±2.5	22.0±2.4	16.8±2.32
Se/Cr	1.56±0.13	1.16±0.09	0.276±0.052
Se/Cs	28.3±2.4	42.0±4.2	26.4±9.9
Se/Dy	456±67	763±139	182±61
Se/Er	794±96	1791±372	262±68
Se/Fe	0.00755±0.00049	0.00919±0.00091	0.00397±0.00052
Se/Gd	416±61	816±166	90.8±26.0
Se/Hg	22.0±2.5	5.94±0.90	5.15±0.66
Se/Ho	2142±273	3772±651	358±36
Se/La	43.4±8.6	58.8±9.7	3.93±1.94
Se/Li	26.0±2.7	32.5±5.7	4.45±1.58
Se/Mn	0.644±0.051	0.859±0.110	0.180±0.055
Se/Mo	4.10±0.53	5.79±0.62	3.23±1.10
Se/Nb	362±72	473±103	183±89
Se/Nd	91.0±13.8	193±39	23.8±6.15
Se/Ni	0.507±0.100	0.509±0.101	0.105±0.024
Se/Pb	1.26±0.21	2.17±0.48	0.397±0.058
Se/Pr	362±62	989±260	81.8±21.2
Se/Rb	0.0646±0.0062	0.0844±0.0078	0.0636±0.0048
Se/Sb	32.4±4.7	18.4±5.9	2.18±0.46
Se/Sc	30.4±3.7	64.3±13.1	58.8±8.7
Se/Sm	461±66	1408±391	110±26
Se/Sn	6.51±1.24	15.3±3.1	0.825±0.287
Se/Tb	3641±614	7039±1219	1056±299
Se/Th	479±83	684±111	42.6±16.4
Se/Ti*	0.591±0.098	0.792±0.154	0.143±0.048
Se/Tl	718±86	656±73	64.3±25.2
Se/Tm	5041±903	8472±2069	961±138



Se/U	429±76	900±165	109±17
Se/Y	135±40	180±41	25.2±6.0
Se/Yb	1023±167	1512±315	561±111
Se/Zn	0.00106±0.00017	0.00119±0.00019	0.00467±0.00037
Se/Zr	38.4±6.8	33.8±12.7	0.624±0.132

M: Arithmetic Mean, SEM: Standard Error of Mean, \*Titanium tools were used for sampling and sample preparation.

Table 4 depicts mean values±standard error of mean (M±SEM) of the ratio to Se of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction in normal, benign hypertrophic and cancerous prostate.

**Table 5:** Ratio of means and the difference between mean values of the Se mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa).

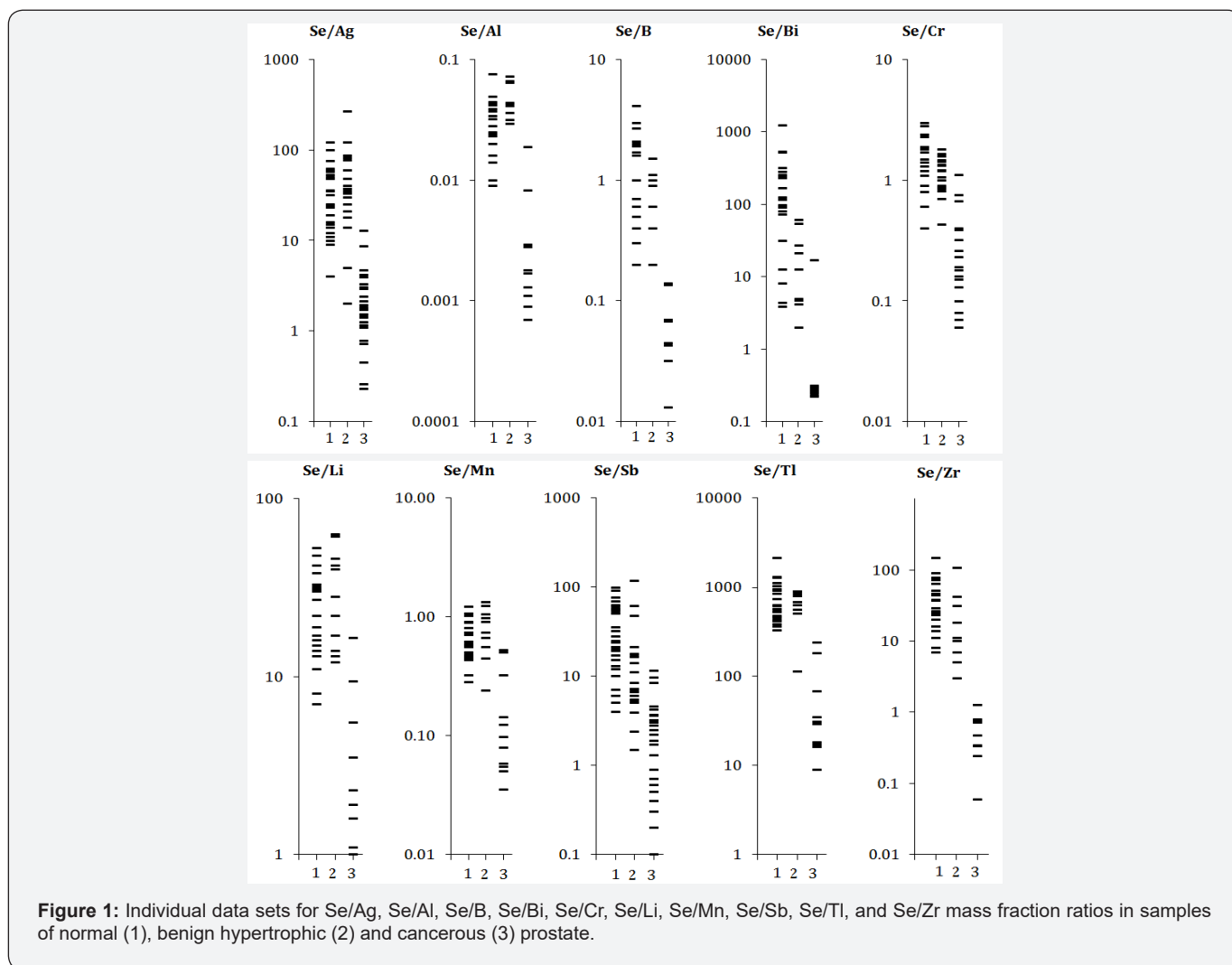
	BPH and N			PCa and N			PCa and BPH		
	Ratio BPH/N	p≤ t-test	p U-test	Ratio PCa/N	p≤ t-test	P U-test	Ratio PCa/BPH	p≤ t-test	P U-test
Se/Ag	1.62	0.117	>0.05	0.072	<b>0.00001</b>	≤ <b>0.01</b>	0.045	<b>0.00022</b>	≤ <b>0.01</b>
Se/Al	1.51	<b>0.019</b>	≤ <b>0.01</b>	0.134	<b>0.00001</b>	≤ <b>0.01</b>	0.088	<b>0.00001</b>	≤ <b>0.01</b>
Se/Au	1.48	0.140	>0.05	0.174	<b>0.00019</b>	≤ <b>0.01</b>	0.117	<b>0.00044</b>	≤ <b>0.01</b>
Se/B	0.54	<b>0.019</b>	≤ <b>0.05</b>	0.050	<b>0.00001</b>	≤ <b>0.01</b>	0.094	<b>0.0015</b>	≤ <b>0.01</b>
Se/Be	1.09	0.547	>0.05	0.058	<b>0.00001</b>	≤ <b>0.01</b>	0.054	<b>0.00001</b>	≤ <b>0.01</b>
Se/Bi	0.09	<b>0.0017</b>	≤ <b>0.01</b>	0.009	<b>0.00077</b>	≤ <b>0.01</b>	0.098	<b>0.031</b>	≤ <b>0.01</b>
Se/Br	1.01	0.960	>0.05	0.203	<b>0.00001</b>	≤ <b>0.01</b>	0.201	<b>0.00001</b>	≤ <b>0.01</b>
Se/Cd	1.94	0.091	>0.05	2.03	<b>0.00072</b>	≤ <b>0.01</b>	1.05	0.859	>0.05
Se/Ce	2.13	<b>0.0072</b>	≤ <b>0.01</b>	0.219	<b>0.00006</b>	≤ <b>0.01</b>	0.103	<b>0.00015</b>	≤ <b>0.01</b>
Se/Co	1.02	0.894	>0.05	0.781	0.171	>0.05	0.764	0.123	>0.05
Se/Cr	0.74	<b>0.017</b>	≤ <b>0.01</b>	0.177	<b>0.00001</b>	≤ <b>0.01</b>	0.238	<b>0.00001</b>	≤ <b>0.01</b>
Se/Cs	1.48	<b>0.013</b>	≤ <b>0.01</b>	0.933	0.849	>0.05	0.629	0.171	>0.05
Se/Dy	1.67	0.065	≤ <b>0.05</b>	0.399	<b>0.0053</b>	≤ <b>0.01</b>	0.239	<b>0.0019</b>	≤ <b>0.01</b>
Se/Er	2.26	<b>0.024</b>	≤ <b>0.01</b>	0.330	<b>0.00008</b>	≤ <b>0.01</b>	0.146	<b>0.0021</b>	≤ <b>0.01</b>
Se/Fe	1.22	0.121	>0.05	0.526	<b>0.00001</b>	≤ <b>0.01</b>	0.432	<b>0.00002</b>	≤ <b>0.01</b>
Se/Gd	1.96	<b>0.042</b>	≤ <b>0.05</b>	0.218	<b>0.00004</b>	≤ <b>0.01</b>	0.111	<b>0.0014</b>	≤ <b>0.01</b>
Se/Hg	0.27	<b>0.00001</b>	≤ <b>0.01</b>	0.234	<b>0.00001</b>	≤ <b>0.01</b>	0.867	0.484	>0.05
Se/Ho	1.76	<b>0.037</b>	≤ <b>0.05</b>	0.167	<b>0.00002</b>	≤ <b>0.01</b>	0.095	<b>0.00037</b>	≤ <b>0.01</b>
Se/La	1.35	0.248	>0.05	0.091	<b>0.00015</b>	≤ <b>0.01</b>	0.067	<b>0.00028</b>	≤ <b>0.01</b>
Se/Li	1.25	0.323	>0.05	0.171	<b>0.00001</b>	≤ <b>0.01</b>	0.137	<b>0.00057</b>	≤ <b>0.01</b>
Se/Mn	1.33	0.097	>0.05	0.280	<b>0.00001</b>	≤ <b>0.01</b>	0.210	<b>0.00006</b>	≤ <b>0.01</b>
Se/Mo	1.41	<b>0.049</b>	≤ <b>0.05</b>	0.788	0.501	>0.05	0.558	0.084	>0.05
Se/Nb	1.31	0.388	>0.05	0.506	0.224	>0.05	0.387	0.090	>0.05
Se/Nd	2.12	<b>0.029</b>	≤ <b>0.05</b>	0.262	<b>0.00012</b>	≤ <b>0.01</b>	0.123	<b>0.0014</b>	≤ <b>0.01</b>
Se/Ni	1.00	0.987	>0.05	0.207	<b>0.00071</b>	≤ <b>0.01</b>	0.206	<b>0.0026</b>	≤ <b>0.01</b>
Se/Pb	1.72	0.105	>0.05	0.315	<b>0.00056</b>	≤ <b>0.01</b>	0.183	<b>0.0044</b>	≤ <b>0.01</b>
Se/Pr	2.73	<b>0.039</b>	≤ <b>0.05</b>	0.226	<b>0.0002</b>	≤ <b>0.01</b>	0.083	<b>0.0059</b>	≤ <b>0.01</b>
Se/Rb	1.31	0.053	>0.05	0.985	0.902	>0.05	0.754	<b>0.029</b>	≤ <b>0.05</b>
Se/Sb	0.57	0.072	>0.05	0.067	<b>0.00001</b>	≤ <b>0.01</b>	0.118	<b>0.012</b>	≤ <b>0.01</b>
Se/Sc	2.12	<b>0.025</b>	≤ <b>0.05</b>	1.93	<b>0.0049</b>	≤ <b>0.01</b>	0.914	0.730	>0.05

Se/Sm	3.05	<b>0.036</b>	<b>≤0.05</b>	0.239	<b>0.00003</b>	<b>≤0.01</b>	0.078	<b>0.0077</b>	<b>≤0.01</b>
Se/Sn	2.35	<b>0.022</b>	<b>≤0.05</b>	0.127	<b>0.00015</b>	<b>≤0.01</b>	0.054	<b>0.00098</b>	<b>≤0.01</b>
Se/Tb	1.93	<b>0.025</b>	<b>≤0.05</b>	0.290	<b>0.0008</b>	<b>≤0.01</b>	0.150	<b>0.00057</b>	<b>≤0.01</b>
Se/Th	1.43	0.155	>0.05	0.089	<b>0.00003</b>	<b>≤0.01</b>	0.062	<b>0.00017</b>	<b>≤0.01</b>
Se/Ti*	1.34	0.284	>0.05	0.242	<b>0.00036</b>	<b>≤0.01</b>	0.181	<b>0.0017</b>	<b>≤0.01</b>
Se/Tl	0.91	0.582	>0.05	0.090	<b>0.00001</b>	<b>≤0.01</b>	0.098	<b>0.00001</b>	<b>≤0.01</b>
Se/Tm	1.68	0.151	>0.05	0.191	<b>0.00016</b>	<b>≤0.01</b>	0.113	<b>0.0046</b>	<b>≤0.01</b>
Se/U	2.10	<b>0.021</b>	<b>≤0.05</b>	0.254	<b>0.00038</b>	<b>≤0.01</b>	0.121	<b>0.00071</b>	<b>≤0.01</b>
Se/Y	1.33	0.438	>0.05	0.187	<b>0.013</b>	<b>≤0.01</b>	0.140	<b>0.0035</b>	<b>≤0.01</b>
Se/Yb	1.48	0.192	>0.05	0.548	<b>0.031</b>	>0.05	0.371	<b>0.016</b>	<b>≤0.01</b>
Se/Zn	1.12	0.614	>0.05	4.41	<b>0.00001</b>	<b>≤0.01</b>	3.92	<b>0.00001</b>	<b>≤0.01</b>
Se/Zr	0.88	0.755	>0.05	0.016	<b>0.00001</b>	<b>≤0.01</b>	0.018	<b>0.028</b>	<b>≤0.01</b>

t-test: Student's t-test, U-test: Wilcoxon-Mann-Whitney U-test, **Bold**: significant differences

The ratios of means and the difference between mean values of the Se/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate are presented in Table 5. Individual data sets for Se/Ag, Se/Al, Se/B, Se/Bi, Se/

Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios in all investigated samples of normal, benign hypertrophic and cancerous prostate, respectively, are shown in Figure 1.



**Figure 1:** Individual data sets for Se/Ag, Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.



**Table 6:** Parameters of the importance (sensitivity, specificity and accuracy) for Se/Ag, Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios for the diagnosis of PCa (an estimation is made for "PCa or normal and BPH prostate").

Mass Fraction Ratio	Upper Limit For PCa <	Sensitivity %	Specificity %	Accuracy %
Se/Ag	8.0	92±5	94±3	92±3
Se/Al	0.0085	90±10	100-3	98±2
Se/B	0.15	100-11	100-3	100-2
Se/Bi	1.0	90±10	100-3	97±3
Se/Cr	0.80	96±4	91±5	93±3
Se/Li	10.0	90±10	94±4	93±4
Se/Mn	0.35	82±12	91±5	89±5
Se/Sb	4.7	92±5	92±4	92±3
Se/Tl	250	100-10	97±3	98±2
Se/Zr	1.5	100-10	100-3	100-2

Table 6 contains parameters of the importance (sensitivity, specificity and accuracy) of Se/Ag, Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios for the diagnosis of PCa calculated in this work.

### Discussion

As was shown by us [14,15,17,18], the use of CRM IAEA H-4 Animal muscle, IAEA HH-1 Human hair, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2Mixed Polish Herbs as certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the trace element contents in these CRMs, measured by us using INAA-LLR and ICP-MS methods, with the certified data (Table 1 & 2) indicates an acceptable accuracy of the results obtained in the present study.

The mean values and standard error of mean (±SEM) were calculated for 43 trace element contents including Se (Table 3), as well as for 42 ratios of Se/trace element mass fractions (Table 4). The mass fraction of Se and other 42 trace elements were measured in all, or a major portion of normal prostate samples. The masses of BPH and PCa samples varied very strong from a few milligrams (sample from needle biopsy material) to 100mg (sample from resected material). Therefore, in BPH and PCa prostates mass fraction ratios of Se/Ag, Se/Co, Se/Cr, Se/Fe, Se/Hg, Se/Rb, Se/Sb, Se/Sc, and Se/Zn were measured in all, or a major portion of samples, while ratios to Se of other trace element content were determined in 21 samples (11 BPH and 10 PCa samples, respectively).

From Table 5, it is observed that in benign hypertrophic tissues the Se/Ag, Se/Au, Se/Be, Se/Br, Se/Cd, Se/Co, Se/Fe, Se/La, Se/Li, Se/Mn, Se/Nb, Se/Ni, Se/Pb, Se/Rb, Se/Sb, Se/Th, Se/Ti, Se/Tl, Se/Tm, Se/Yb, Se/Zn, and Se/Zr mass fraction ratios not differ from normal levels, but the mass fraction ratios of

Se/Al, Se/Ce, Se/Cs, Se/Dy, Se/Er, Se/Gd, Se/Ho, Se/Mo, Se/Nd, Se/Pr, Se/Sc, Se/Sm, Se/Sn, Se/Tb, and Se/U are higher, while the mass fraction ratios of Se/B, Se/Bi, Se/Cr, and Se/Hg are significantly lower. In cancerous tissue the all Se/trace element mass fraction ratios investigated in the study are significantly lower, than in BPH and normal prostate, with the exception of Se/Cd, Se/Co, Se/Cs, Se/Hg, Se/Mo, Se/Nb, Se/Rb, Se/Sc, Se/Yb, and Se/Zn ratios.

Analysis of the mass fraction ratios for trace element in prostate tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen (PSA) serum test [77,78]. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In BPH and PCa cases we analyzed a part of the material obtained from a puncture trans rectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Se/trace element mass fraction ratios for the diagnosis of PCa. As is evident from Table 5 and, particularly, from individual data sets (Figure 1), the for Se/Ag, Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 8.0 is the value of Se/Ag mass fraction ratio assumed to be the upper limit for PCa (Figure 1) and an estimation is made for "PCa or intact and BPH tissue", the following values are obtained:

$$\text{Sensitivity} = \{ \text{True Positives (TP)} / [\text{TP} + \text{False Negatives (FN)}] \} \cdot 100\% = 92 \pm 5\%;$$

$$\text{Specificity} = \{ \text{True Negatives (TN)} / [\text{TN} + \text{False Positives (FP)}] \} \cdot 100\% = 94 \pm 3\%.$$

$$\text{Accuracy} = [(\text{TP} + \text{TN}) / (\text{TP} + \text{FP} + \text{TN} + \text{FN})] \cdot 100\% = 92 \pm 3\%.$$

The number of people (samples) examined was taken into account for calculation of confidence intervals [79]. In other words, if Se/Ag mass fraction ratio in a prostate biopsy sample is lower 8.0, one could diagnose a malignant tumor with an accuracy 92±3%. Thus, using the Se/Ag mass fraction ratio-test makes it possible to diagnose cancer in 92±5% cases (sensitivity). The same way parameters of the importance (sensitivity, specificity and accuracy) of for Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios for the diagnosis of PCa were calculated (Table 6).

### Conclusion

The combination of nondestructive INAA-LLR and destructive ICP-MS methods is satisfactory analytical tool for the precise determination of 43 trace element mass fractions in the tissue samples of normal, BPH and carcinomatous prostate

glands. The sequential application of these methods allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. It was observed that the ratio to Se of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Dy, Er, Fe, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Rb, Sb, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. It was proposed to use the Se/Ag, Se/Al, Se/B, Se/Bi, Se/Cr, Se/Li, Se/Mn, Se/Sb, Se/Tl, and Se/Zr mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

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