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Method and portable facility for measurement of trace element concentration in prostate fluid samples using radionuclide-induced energy-dispersive X-ray fluorescent analysis

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Abstract A facility and method for ^{109}Cd radionuclide-induced energy-dispersive X-ray fluorescent (EDXRF) were developed to determine the Fe, Zn, Br, Rb, and Sr concentrations in the specimens of human prostatic fluid. Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18–82 years) with apparently normal prostates using standard rectal massage procedure. Mean values ($M \pm SEM$) for concentration of trace elements (mg L^{-1}) in human prostate fluid were: Fe 9.04 ± 1.21, Zn 573 ± 35, Br 3.58 ± 0.59, Rb 1.10 ± 0.08, and Sr $\leq$ 0.76. It was shown that the results of trace element analysis in the micro-samples (20 μL) are sufficiently representative for assessment of the Fe, Zn, Br, and Rb concentration in the prostate fluid. The facility for ^{109}Cd radionuclide-induced EDXRF is comparatively compact and can be located in close proximity to the site of carrying out the massage procedure. The means of Zn and Rb concentration obtained for prostate fluid agree well with median of reported means. For the first time, the Fe and Br concentrations, as well as the upper limit of the Sr concentration, were determined in the human prostate fluid.

Keywords Radionuclide-induced energy-dispersive X-ray fluorescent analysis · Trace elements · Human prostate · Prostate fluid · ^{109}Cd

1 Introduction

The first finding of remarkable high level of Zn concentration in human prostatic fluid was reported in the beginning of 1960s [1]. Analyzing prostatic secretion expressed from prostate of 8 apparently healthy men aged 25–55 years; it was found that Zn concentration varied in range from 300 to 730 mg L^{-1}. After this finding, several investigators have suggested that the measurement of Zn level in expressed prostatic fluid may be useful as a marker of prostate secretory function [2]. It promoted a more detailed study of prostatic fluid Zn concentration in healthy subjects and in those with different prostate diseases: chronic prostatitis, benign prostatic hyperplasia (BPH), and prostate cancer (PCa). A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication [3].

The studies of Zn in prostatic fluid expressed from normal and neoplastic prostate glands were done in the second half of 1970s in the Medical Radiological Research Center in Obninsk, Russia (MRRC), using nondestructive nuclear analytical methods. Nondestructive methods of analysis avoid the possibility of changing the Zn content in the studied samples during sample preparation, which allowed for the first time to obtain reliable results. In particular, it was shown that the average concentration of Zn in prostatic fluid in cancerous gland is 15–17 times lower than in healthy or hyperplastic prostates [3]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which was to determine the Zn concentration in the sample of expressed prostatic fluid. For the first time, it was proposed to use energy-dispersive X-ray fluorescence (EDXRF) to determine Zn concentration in the sample of

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expressed prostatic fluid. The method has been successfully used in clinical trials of MRRC, and it was patented in Russia [4]. All data on the new method of diagnosing PCa were classified by Ministry of Health of the USSR, and publications were made possible only in the 90s, after the radical political changes in the country. Publications in international scientific journals [3] and presentations at international medical conferences [5, 6] have stimulated the interest in new approaches for early diagnosis of PCa based on the phenomenon of a sharp reduction in the ability of the prostate fluid to accumulate Zn after malignant transformation of gland. Currently, work in this direction is carried out in several research centers and hospitals in the USA [7, 8] and other countries [9]. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to gained experience in a critical assessment of the capacity of the PSA serum test [10].

In order to determine Zn concentration in the sample of expressed prostatic fluid, one can use commercially available systems of different analytical methods like EDXRF, AAS, ICP-AES, ICP-MS, and others. Each of these methods has advantages and disadvantages, the discussion of which is beyond the scope of this work.

To determine Zn concentration in the sample of expressed prostatic fluid, we have chosen radionuclide-induced EDXRF. A simple inexpensive device that uses a miniature source of exciting radiation from ^{109}Cd radionuclide was developed by us. Our decision was based on two reasons. First, large radiology centers usually have a Spectrometric Laboratory for the measurement of radiations, including photons of 1–100 keV, and such equipment eliminates one's need additional expensive purchases to use EDXRF. Second, the volume of expressed prostatic fluid sample as usual does not exceed a few tens of microliters. Thus, the ultimate goal of research conducted in the MRRC was to develop a method for measurement of Zn concentration in the sample with volume equal to one drop (nearly 20 µL) of prostatic fluid and to create a miniature X-ray fluorescence device available for PCa screening. We believe that the creation of such a device is possible on the basis of EDXRF analysis using a miniature radionuclide sources. Therefore, EDXRF analysis experience using ^{109}Cd source to determine Zn concentration in the sample of expressed prostatic fluid might be useful for further development of PCa screening.

Our previous studies found that not only Zn but many others trace elements may be useful as tumor markers [11–22]. Thus, this work had three aims. The first one was to present the design of the method and apparatus for micro-analysis of Zn and some other trace elements in the samples of expressed prostatic fluid using EDXRF with radionuclide source ^{109}Cd. The second aim was to evaluate the quality of obtained results, and the third was to assess the concentration of Br, Fe, Rb, Sr, and Zn in expressed prostatic fluid of normal prostate gland.

The study was approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

2 Experimental section

Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18–82 years) with apparently normal prostates by qualified urologists in the Urological Department of the Medical Radiological Research Centre using standard rectal massage procedure. Subjects were asked to abstain from sexual intercourse for 3 days preceding the procedure. Specimens of expressed prostatic fluid were obtained in sterile containers which were appropriately labeled. Twenty µL (microliters) of fluid were taken by micropipette from every specimen for trace element analysis, while the rest of the fluid was used for cytological and bacteriological investigations. The chosen 20 µL of the expressed prostatic fluid was dropped on 11.3-mm-diameter disk made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in an exsiccator at room temperature. Then, the dried sample was covered with 4 µm Dacron film and centrally pulled onto a Plexiglas cylindrical frame (Fig. 1).

To determine concentration of the elements by comparison with a known standard, aliquots of solutions of commercial, chemically pure compounds were used for a device calibration [23]. The standard samples for calibration were prepared in the same way as the samples of prostate fluid. Because there was no available liquid certified reference material (CRM), ten subsamples of the powdery CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. Every CRM subsample weighing about 3 mg was applied to the

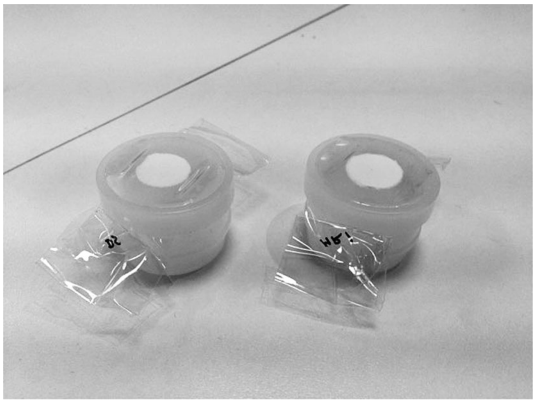

Fig. 1 Dried samples of prostate fluid on filter paper disks fixed on the Scotch tape pieces centrally pulled onto a Plexiglas cylindrical frame

piece of Scotch tape serving as an adhesive fixing backing. An acrylic stencil made in the form of a thin-walled cylinder with 11.3-mm inner diameter was used to apply the subsample to the Scotch tape. The polished-end acrylic pestle which is a constituent of the stencil set was used for uniform distribution of the subsample within the Scorch surface restricted by stencil inner diameter. When the subsample was slightly pressed to the Scotch adhesive sample, the stencil was removed. Then, the subsample was covered with 4-µm Dacron film. Before the sample was applied, pieces of Scotch tape and Dacron film were weighed using analytical balance. Those were again weighed together with the sample inside to determine the subsample mass precisely.

The facility for radionuclide-induced energy-dispersive X-ray fluorescence included an annular ^{109}Cd source with an activity of 2.56 GBq, Si(Li) detector with electric cooler, and portable multi-channel analyzer combined with a PC. Its resolution was 270 eV at the 6.4 keV line. The facility functioned as follows. Photons with a 22.1 keV ^{109}Cd energy are sent to the surface of a specimen analyzed inducing the fluorescence K_α X-rays of trace elements. The fluorescence irradiation got to the detector through a 10-mm-diameter collimator to be recorded.

The duration of the Zn concentration measurement was 10 min. The duration of the Zn concentration measurement together with Br, Fe, Rb, and Sr was 60 min. The intensity of K_α-line of Br, Fe, Rb, Sr, and Zn for samples and standards was estimated on calculation basis of the total area of the corresponding photopeak in the spectra. The trace element concentration was calculated by the relative way of comparing between intensities of K_α-lines for samples and standards. Following the assumption that the surface density of dried samples and standards meets the criterion of "thin sample" [24, 25], the trace element concentration in the sample (C_s) can be calculated from the following simple relation:

$$C_s = (Q_{\mathrm{st}} \times N_{\mathrm{s}})/(m_{\mathrm{s}} \times N_{\mathrm{st}}), \qquad (1)$$

where N_{s} and N_{st} are trace element K_α-line intensity (counts per minute) in the sample and standard spectra, respectively; m_{s} is the sample mass (kg) or volume (L); Q_{st} is trace element content in the standard (mg).

The relative counting statistical uncertainty ("statistical error") and the detection limit (*DL*) depend on mass of the sample and the duration of measurement. The "statistical error" of zinc K_α-line measurement for 10 min of most prostate fluid samples did not exceed 3.0 %. The DL (mg L^{-1}) for the Fe, Br, Rb, Sr, and Zn determination in 20 µL sample of dry prostate fluid sample with 60 min measurement was about 2.0, 0.5, 0.3, 0.1, and 1.0, respectively. The detection limit of element was calculated as:

$$\mathrm{DL} = (3\sqrt{N_{\mathrm{b}}})/RF, \qquad (2)$$

where N_{b} is the background intensity in K_α-line area of corresponding element and RF is a response function for this element:

$$RF = (N_{\mathrm{st}} - N_{\mathrm{b}})/C_{\mathrm{st}}, \qquad (3)$$

where N_{st} is the intensity of corresponding K_α-line for the standard and C_{st} is trace element concentration in the standard (mg L^{-1}).

Using the Microsoft Office Excel program to provide a summary of statistical results, the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, and percentiles with 0.025 and 0.975 levels were calculated for all the chemical element concentrations obtained.

3 Results and discussion

Figures 1 and 2 depict the dried samples of prostate fluid prepared for the analysis and the facility for Fe, Zn, Br, Rb, and Sr concentration measurement in prostate fluid samples by the ^{109}Cd radionuclide-induced EDXRF, respectively.

Table 1 presents our calculation which needs for an estimation of the maximum surface density of the "thin sample" for ^{109}Cd radionuclide-induced EDXRF analysis of Fe content in samples of dry and wet prostate fluid using published data on fluid chemical composition [3, 4, 7, 26–32] and photon cross sections [33]. Table 2 shows ^{109}Cd

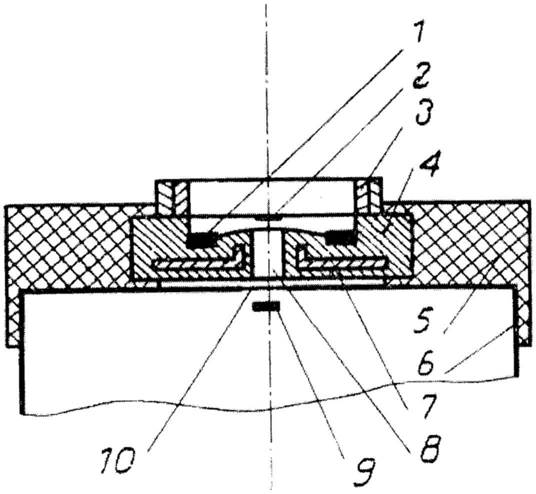

Fig. 2 The Facility for analysis of the Fe, Zn, Br, Rb, and Sr concentration in prostate fluid micro-samples (20 µL) by the radionuclide-induced EDXRF. *1* 2.56 GBq annular ^{109}Cd source; *2* prostate fluid sample; *3* Plexiglas cylindrical frame for holding samples in the measurement position; *4* shield is made of pure aluminum; *5* Plexiglas; *6* detector cryostat wall; *7* tantalum shield, *8* collimator aperture 10-mm diameter; *9* detector crystal; *10* beryllium foil

Table 1 Mass fraction of the chemical elements and values of mass attenuation coefficients used to calculate maximum surface density of the "thin sample" during EDXRF analysis of Fe concentration in wet and dry prostate fluid

Element	Concentration and mass fraction $(c_i)^a$		μ_i^{1b}	μ_i^{2b}	$\overline{\mu}_i^c$ (cm^2 g^{-1})	c_i $\overline{\mu}_i$ (cm^2 g^{-1})	
	Wet prostate fluid (g ml^{-1})	Dry prostate fluid (g g^{-1})	22.1 keV ^{109}Cd (cm^2 g^{-1})	6.40 keV K_α Fe (cm^2 g^{-1})		Wet tissue of prostate	Dry tissue of prostate
H	0.1000000	0.0600000	0.365	0.399	1.13	0.112900	0.067740
C	0.0370000	0.5000000	0.375	8.97	9.72	0.359640	4.860000
N	0.0110000	0.1400000	0.512	14.7	15.72	0.172964	2.201360
O	0.8420000	0.1800000	0.695	22.6	23.99	20.199580	4.318200
Na	0.0030000	0.0400000	1.583	57.8	60.97	0.182898	2.438640
Mg	0.0004860	0.0069000	2.100	76.8	81.00	0.039366	0.558900
P	0.0000340	0.0004800	3.999	144	152.00	0.005168	0.072959
S	0.0014000	0.0200000	5.002	177	187.00	0.261806	3.740080
Cl	0.0010000	0.0100000	6.421	225	237.84	0.237842	2.378420
K	0.0018000	0.0250000	8.100	273	289.20	0.520560	7.230000
Ca	0.0008000	0.0100000	9.706	318	337.41	0.269930	3.374120
Mn	0.0000010	0.0000143	16.97	61.2	95.14	0.000095	0.001361
Fe	0.0000090	0.0001290	19.41	70.9	109.72	0.000987	0.014154
Cu	0.0000004	0.0000057	25.68	95.2	146.56	0.000059	0.000835
Zn	0.0004500	0.0064290	28.42	106	162.84	0.073278	1.046898
Br	0.0000030	0.0000429	40.62	163	244.24	0.000733	0.010478
Rb	0.0000020	0.0000286	46.15	191	283.30	0.000567	0.008102
Sr	0.0000010	0.0000143	49.27	208	306.54	0.000307	0.004384
Cd	0.0000001	0.0000014	14.45	408	436.90	0.000044	0.000612
Σ c_i	0.999	0.999			Σ $c_i \cdot \mu_i$	22.44	32.33

ψ is a reflection angle of detection beam (90°)

[a] Mean concentrations for prostate tissue are calculated based on reference data [3, 4, 7, 25–31]

[b] Values of μ_i^1 and μ_i^2 are calculated from data of Marenkov et al. [32]

[c] $\overline{\mu} = (\mu_i^1/\mathrm{Sin}\vartheta) + (\mu_i^2/\mathrm{Sin}\psi)$, where ϑ is an incidence angle of excitation beam (30°)

Table 2 EDXRF data Br, Fe, Rb, Sr, and Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg kg^{-1}, dry mass basis)

Element	Certified values			This work results
	Mean	95 % confidence interval	Type	Mean ± SD
Fe	49	47–51	C	48 ± 9
Zn	86	83–90	C	90 ± 5
Br	4.1	3.5–4.7	C	5.0 ± 1.2
Rb	18	17–20	C	22 ± 4
Sr	0.1	–	N	<1

Mean arithmetical mean, *SD* standard deviation, *C* certified values, *N* noncertified values

radionuclide-induced EDXRF data for Fe, Zn, Br, Rb, and Sr mass fractions in subsamples of certified reference materials IAEA H-4 (animal muscle) and the certified values of this material. Table 3 represents some basic statistical parameters (arithmetic mean, standard deviation, relative standard deviation, standard error of mean, minimal and maximal values, median, and percentiles with 0.025 and 0.975 levels) of Fe, Zn, Br, Rb, and Sr concentration (mg L^{-1}) in human prostate fluid. The comparison of our results with published data for Fe, Zn, Br, Rb, and Sr concentration in the prostate fluid expressed out of a normal human gland [4, 34, 35] is shown in Table 4. The estimation of repeatability of the ^{109}Cd radionuclide-induced EDXRF results for Fe, Zn, Br, Rb, and Sr concentrations determined in 3 subsamples taken from few specimens of prostate fluid is shown in Table 5.

Table 3 Some basic statistical parameters of Fe, Zn, Br, Rb, and Sr concentration (mg L^{-1}) in human prostate fluid

Element	Mean	SD	RSD (%)	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Fe	9.04	7.28	80.5	1.21	1.27	39.8	7.84	1.29	21.3
Zn	573	202	35.3	28	253	948	552	260	941
Br	3.58	3.31	92.5	0.59	0.16	10.0	1.63	0.19	9.16
Rb	1.10	0.51	46.4	0.08	0.38	2.45	1.03	0.41	2.36
Sr	≤0.76	–	–	–	<0.1(DL)	3.44	–	–	–

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Per.* 0.025 percentile with 0.025 level, *Per.* 0.975 percentile with 0.975 level, *DL* detection limit

Table 4 Median, minimum, and maximum value of means of chemical element concentration (mg L^{-1}) in human prostatic fluid according to data from the literature

Element	Published data [References]			This work results M ± SD
	Median of means $(n)^{a}$	Minimum of means M or M ± SD, $(n)^{b}$	Maximum of means M ± SD, $(n)^{b}$	
Fe	–	–	–	9.04 ± 7.28
Zn	453 (19)	47.1(-) [32]	9870 ± 10130 (11) [33]	573 ± 202
Br	–	–	–	3.58 ± 3.31
Rb	2.26 (1)	1.11 ± 0.57 (15) [4]	2.35 ± 1.85 (11) [4]	1.10 ± 0.51
Sr	–	–	–	≤0.76

M arithmetic mean, *SD* standard deviation, $(n)^{a}$ number of all references, $(n)^{b}$ number of samples

Distribution of trace elements on filter paper disk in the samples prepared for ^{109}Cd radionuclide-induced EDXRF can be inhomogeneous. To reverse the effect of inhomogeneous distribution of the element on the outcome of the analysis, we have used annular ^{109}Cd source, providing an even distribution of exciting radiation at the surface of the sample (Fig. 2). In addition to photons with 22.16 keV (Ag $K_{\alpha1}$), 21.99 keV (Ag $K_{\alpha2}$), 24.9 keV (Ag $K_{\beta1}$), and 25.5 keV (Ag $K_{\beta2}$), a ^{109}Cd source emits gamma-quanta with energy 88 keV (output 4.2 %). To protect the crystal detector from a direct hit of 88 keV gamma-quanta, we designed a tantalum shield (Fig. 2). The choice of a suitable geometry in «source—sample—detector crystal » was based on the results of a special study [36].

By definition, the "thin sample" self-absorption does not exceed 10 % [24, 25]. In the process of ^{109}Cd radionuclide-induced EDXRF, samples and standards meet the criterion of "thin sample," provided their surface density ($\rho \cdot d$) did not exceed the following value:

$$\rho \cdot d \leq \frac{0.1}{\Sigma c_i \cdot \overline{\mu_i}}, \qquad (4)$$

where ρ is mass density of the analyzed sample, d is a sample thickness, c_i is a relative individual mass fraction of the element in the sample, $\overline{\mu}_i$ is a mean value of the total mass attenuation coefficient of exciting and characteristic radiation with regard to geometry in the system of "source-sample-detector" [24, 25, 36]. The value of $\sum c_i \cdot \mu_i$ is 22.4 and 33.2 cm^2 g^{-1} for Fe (element with lowest characteristic X-ray radiation 6.4 keV measured in the study) in the wet and dry prostate fluid sample, respectively (Table 1). It follows that samples of dry prostate fluid meet the criterion of "thin sample" only in case if their surface density does not exceed 0.0030 g cm^{-2}. The sample mass of dry prostate fluid which fills the backing area of 1 cm^2 (11.3-mm-diameter filter paper disk) should not exceed 3.0 mg. The content of H_2O in the prostate fluid equals 93 % [27]. Therefore, dry mass of 20 µL prostate fluid sample (1.4 mg) is under the limit. We can accept that chemical matrix of dried animal muscle is similar to matrix of dried prostate fluid (both are mainly proteins). Thus, the results of calculation showed that the prepared prostate fluid and CRM IAEA H-4 samples conformed to the criterion of "thin sample." In its turn, the conformity of samples and standards with the criterion of "thin sample" confirmed the competence of using the chosen way for calculation of Fe contents in the analyzed samples. Energies of K_α X-rays of Zn, Br, Rb, and Sr are higher than K_α Fe. It means that the prepared samples complied with the criterion of "thin sample" for the measurement of Zn, Br, Rb, and Sr contents too.

Good agreement of the Fe, Zn, Br, Rb, and Sr contents analyzed by ^{109}Cd radionuclide-induced EDXRF with the certified data of CRM IAEA H-4 (Table 2) indicates an

Table 5 Repeatability of the EDXRF results for Fe, Zn, Br, Rb, and Sr concentration (mg L^{-1}) in 3 subsamples taken from the specimens of the prostate fluid

Element	Specimen No.	Subsample			Mean	SD	RSD (%)	Mean RSD (%)
		No. 1	No. 2	No. 3				
Fe	55	12.3	10.2	14.2	12.2	2.00	16.4	11.0
	59	17.3	16.0	18.7	17.3	1.35	7.8	
	61	7.3	7.1	7.6	7.33	0.25	3.4	
	62	7.3	8.7	8.8	8.27	0.84	10.1	
	65	14.0	19.8	16.5	16.8	2.91	17.4	
Zn	55	740	552	628	640	94.6	14.8	8.3
	59	549	614	493	552	60.6	11.0	
	61	566	555	578	566	11.5	2.0	
	62	593	662	625	627	34.5	5.5	
	65	458	533	470	487	40.3	8.3	
Br	55	1.46	1.57	1.30	1.44	0.14	9.4	29.9
	59	5.46	7.66	3.26	5.46	2.20	40.3	
	61	3.57	5.83	1.32	3.57	2.26	63.1	
	62	8.92	7.74	10.0	8.89	1.13	12.7	
	65	8.53	8.53	5.45	7.50	1.78	23.7	
Rb	55	1.35	1.03	1.67	1.35	0.32	23.7	22.3
	59	1.31	1.56	1.06	1.31	0.25	19.1	
	61	0.44	0.38	0.85	0.56	0.26	46.0	
	62	0.41	0.41	0.49	0.44	0.05	10.6	
	65	1.03	1.15	0.90	1.03	0.13	12.2	
Sr	55	<DL	<DL	<DL	–	–	–	–
	59	1.33	1.29	1.37	1.33	0.04	3.0	
	61	<DL	<DL	<DL	–	–	–	
	62	<DL	<DL	<DL	–	–	–	
	65	1.97	3.44	1.50	2.30	1.01	43.9	

Mean arithmetical mean, *SD* standard deviation, *RSD* relative standard deviation, *DL* detection limit

acceptable accuracy of the results obtained in the study of trace elements of the prostate fluid presented in Tables 3, 4 and 5.

The ^{109}Cd radionuclide-induced EDXRF allowed the assessment of the mean concentrations or an upper limit of concentration of 5 trace elements (Fe, Zn, Br, Rb, and Sr) in the micro-samples (20 μL) of prostate fluid. The contents of Fe, Zn, Br, and Rb were measured in all or a major portion of prostate fluid samples. Mean values ($M \pm SEM$) for concentrations (mg L^{-1}) of 4 trace elements in the prostate fluid between ages 18–82 years were: Fe 9.0 ± 1.2, Zn 573 ± 28, Br 3.58 ± 0.59, and Rb 1.10 ± 0.08 (Table 3). The Sr concentration was determined in a few samples. The possible upper limit of the mean ($\leq M$) for this trace element was calculated as the average concentration, using the value of the detection limit ($DL = 0.1$ mg L^{-1}) instead of the individual value when the latter was found to be below the DL:

$$\leq M = \left(\sum_{i}^{n_i} C_i + DL \cdot n_j \right) / n \tag{5}$$

where c_i is the individual value of the trace element mass fraction in sample $-i$, n_i is number of samples with mass fraction higher than the DL, n_j is number of samples with mass fraction lower than the DL, and $n = n_i + n_j$ is number of samples that were investigated. The upper limit of mass fraction of this trace element was: Sr ≤ 1.05 mg L^{-1}.

The mean values concentrations and all selected statistical parameters were calculated for Fe, Zn, Br, and Rb in the prostate fluid of apparently healthy males aged 18–82 years (Table 3). The mean of Zn concentration obtained for prostate fluid, as shown in Table 4, agrees well with median of means cited by other researches [1–4, 9, 29–32, 34, 35]. The mean of Rb concentration obtained for prostate fluid agrees well with our data reported 35 years ago [4]. No published data referring to Fe, Br, and Sr concentrations in prostate fluid were found.

Uncertainties expressed as mean relative standard deviation of the ^{109}Cd radionuclide-induced EDXRF results from Fe, Zn, Br, and Rb determination in 3 subsamples of 5 prostate fluid specimens were 11, 8, 30, and 22 %, respectively (Table 5). These values of result uncertainties are 2–7 times lower than *RSD* connected to individual variations of the Fe, Zn, Br, and Rb concentrations in prostate fluid of 81, 35, 93, and 46 %, respectively (see Table 3). It means that developed method of Fe, Zn, Br, and Rb measurement in micro-specimens of prostate fluids is acceptable for using in clinical studies [37, 38].

The ^{109}Cd radionuclide-induced EDXRF analysis developed to determine the Fe, Zn, Br, Rb, and Sr concentrations in prostate fluid samples is a nondestructive method. It has a great advantage over destructive analytical methods. Almost all analytical methods used for chemical element measurements in prostate fluid were based on investigation of processed fluid with a goal to destroy and remove organic matrix. In such studies, prostate fluid samples were acid digested or dried and then ashed before analysis. There is evidence that certain quantities of chemical elements are lost as a result of such treatment [39–41]. Thus, when using destructive analytical methods, it is necessary to control for the losses of trace elements, for complete acid digestion of the sample, and for the contaminations by trace elements during sample decomposition, which needs adding some chemicals. It is possible to avoid these procedures using nondestructive methods, including the ^{109}Cd radionuclide-induced EDXRF.

The ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostate fluid is micro-method because sample volume 20 μL (one drop) is quite enough for analysis. It is another advantage of the method. Amount of human prostatic fluid collected by massage of the normal prostate is usually in range 100–500 μL [25] but in a pathological state of gland, particularly after malignant transformation, this amount may be significantly lower. Therefore, the micro-method of ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostate fluid is available for using in clinical studies.

4 Conclusion

The facility and method for ^{109}Cd radionuclide-induced EDXRF were developed to determine the Fe, Zn, Br, Rb, and Sr concentrations in the micro-samples (20 μL) of expressed prostate fluid. The results of trace element analysis in the micro-samples are sufficiently representative for assessment of the Fe, Zn, Br, and Rb concentration in the prostate fluid. The ^{109}Cd radionuclide-induced EDXRF analysis of Fe, Zn, Br, Rb, and Sr concentration in the prostate fluid requires no preliminary sample preparation; it is nondestructive and takes not more than 10 min for Zn and 60 min for all trace element measurement. The facility for ^{109}Cd radionuclide-induced EDXRF is comparatively compact and can be located in close proximity to the site of carrying out the massage procedure. The means of Zn and Rb concentration obtained for prostate fluid agree well with median of reported means. For the first time, the Fe, Br, and Sr concentrations were determined in the human prostate fluid.

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