

**REVIEW**  
FOR THE COMPETITION FOR AWARDING THE ACADEMIC POSITION  
"ASSOCIATE PROFESSOR"  
IN THE FIELD OF HIGHER EDUCATION 7. "HEALTH AND SPORTS",  
BY PROFESSIONAL FIELD 7.1. "MEDICINE" AND  
SCIENTIFIC SPECIALTY "CLINICAL LABORATORY",  
ANNOUNCED IN SATE JOURNAL, NO. 59 / 26.07.2022,  
FOR THE NEEDS OF DEPARTMENT OF CLINICAL LABORATORY,  
FACULTY OF MEDICINE, MU - VARNA, (0.25 full-time position)

One admitted candidate for the competition

**Irena Dimitrova Ivanova, MD, PhD**

from

**Prof. Dr. Margaritka Ivanova Boncheva, MD,**

Head of Clinical Laboratory

at UMBAL "Prof. Dr. Alexander Chirkov" EAD-Sofia, internal member of the scientific jury,  
by Order No. R-109-381/26.09.2022. of the Rector of MU-Varna

For this competition, Irena Dimitrova Ivanova, MD, PhD, has submitted all the necessary documents in accordance to the national requirements defined in the Law on the Development of the Academic Staff in the Republic of Bulgaria and Regulations for Development of Academic Staff at MU-Varna. The documents are carefully arranged and numbered, as well as Academic report from MU-Varna, No. 638/16.08.2021.

1. Brief biographical information.

Irena Dimitrova Ivanova was born on 15 May 1978 in Ruse. In 2003 she graduated with a master's degree in the specialty "Medicine" and a professional qualification "Master - doctor". In 2009 she acquired the "Clinical Laboratory" medical specialty and in 2016 she obtained the educational and scientific degree "PhD" in Clinical laboratory discipline. Dr. Ivanova also obtained a Master's degree in Public Health and Health Management. She worked successively as: until 2005 as a resident at the Internal department of the Pazardzhik General Hospital, 2005-2009 as specialist in the clinical laboratory of the UH "St. Ivan Rilski" - Sofia, 2009-2010 she is a specialist at the same workplace, and since 2010 until now she is the head of the same laboratory. Dr. Irena Ivanova's academic development is consistent. She started in 2016, and until 2021 she is a part-time assistant in the discipline "Clinical Laboratory" at MU-

Sofia, Faculty of Medicine, Department of Clinical Laboratory. Dr. Ivanova teaches medical students in Bulgarian and English. From 2016 until now, she is a "part-time teacher" in the disciplines " Medical chemistry" and "Clinical Practicum" at Southwestern University "Neofit Rilski" - Blagoevgrad, Faculty of Science and Mathematics, Department of Chemistry. From 2021-2022 she is a "part-time lecturer" in "Clinical chemistry" at Sofia University "St. Kliment Ohridski" - Sofia, Faculty of Chemistry and Pharmacy, Department of Analytical Chemistry.

## 2. Research activity

### **Quantitative criteria:**

The candidate has presented the following scientific papers:

- (A1) Dissertation on the topic: "Copper status - laboratory aspects and clinical application in some pathological conditions", defended 2016
- (V 3) Monographic habilitation thesis: "Copper metabolism - physiological mechanisms and clinical application", 2022, ISBN: 978-619-7491-50-0
- (G 5-9) Author of a collective monograph chapter – Chapter 42 – "ATP7B and Alzheimer Disease". In Kerkar N, Roberts EA editors. In: Clinical and Translational Perspectives on Wilson Disease. Academic Press;2019. p.427-36.
- The 3 articles related to the doctoral dissertation are shown (G7. 1, 2 and 3)
- 21 titles of scientific works are presented, which are now subject to review (G7 – 12 publications, G8 – 8 publications, and one supplement. Out of 21 titles, 14 are full-text publications and 7 are conference papers. In the presented scientific works, Dr. Ivanova is the first author in 10 of the publications, the second author in 4, the third author in 2, the fourth author in 3, the fifth author in 1, and the seventh author in 1 publication. It is obvious that Dr. Irena Ivanova has made a significant and leading contribution in her scientific developments.
- (D) Citations: Reference of the National Library "St. St. Cyril and Methodius" (from 12.08.2022) found 42 citations of scientific works of Dr. Irena Ivanova. In the Academic reference of MU-Varna, 4 citations are found (which overlap in the reference of the "St. St. Cyril and Methodius" library").
- IP : According to the Academic Reference of MU-Varna dated 16.08.2022, for the journals in which Dr. Ivanova published (and the corresponding years), currently IP 7,869 are collected.

Minimum required points of NACID by groups of indicators for the academic position " Assistant Professor " and points of Dr. Ivanova.

Group of indicators	Content	Minimum number of points for "Assistant Professor"	Points
A	Indicator 1	50	50
B	Indicator 2	-	-
V	Indicator 3	100	100
G	Sum of indicators 5 to 9	200	225.55
D	Sum of indicators 10 to 12	50	60
E	Sum of the indicators from 13 to the end	-	-
Total points		400	<b>435.55</b>

Dr. Irena Ivanova has participated in a total of 29 congresses and conferences - 14 national and 15 - international. In all, she contributed oral presentations and/or abstracts in poster sessions, with all abstracts printed as conference papers in the Book of Abstracts. In the specialized and representative journals of the IFCC and EFLM, abstracts of Dr. Ivanova's participation in poster sessions or reports at 6 international forums have been published.

#### **Scientific contributions.**

Dr. Ivanova is a scientist with extensive knowledge in clinical laboratory and clinical chemistry. Despite the prevailing scientific interest in microelement analysis of blood and its relation to some congenital and neurodegenerative diseases, there are significant studies of the pre-analytical phase of laboratory analyses, research of the stress state, of liver pathology and kidney diseases.

Four areas stand out in Dr. Irena Ivanova's scientific output:

- Clinical-laboratory aspects in trace elements analysis (A1, G7.9, G7.11, G8.1, G8.3, G8.6, G8.7, G9.1).
- Conditions with copper dyshomeostasis (G7.2, G7.5, G7.6, G7.7, G8.3, G8.7, G9.1).
- Reference limits for serum copper and determination of biological variations in healthy Bulgarians (A.1, D7.1, D7.9, D8.8).
- Study of stress markers; new markers in liver pathology (NAFLD) and in kidney diseases; micronutrients and vitamins (G7.3, G7.4, G7.8, G7.10, G7.12, G8.2, G8.4, G8.5).

**Clinical-laboratory aspects in trace elements analysis (A1, G7.9, G7.11, G8.1, G8.3, G8.6, G8.7, G9.1):**

Dr. Ivanova did extensive research on the trace element copper. Contributions in methodology reflect the pre-analytical stage in the study in various biological matrices; role of the type of test tubes in which material is taken for zinc and copper testing; validation of the methodologies with Atomic Absorption Spectroscopy (AAS) for the quantitative study of copper; verification of the immunoturbidimetric method for determination of ceruloplasmin (copper transporter protein) in serum. For the first time in our country, zinc levels in saliva were measured, recommending standardization of the methodology in an international aspect and validation of the individual steps in the analysis. Regarding the methodology for the analysis of ceruloplasmin, Dr. Ivanova's contribution is the study of the enzymatic activity of ceruloplasmin (as ferroxidase) in Bulgarians and the contribution to the data of other authors. This research is important because it shows the physiological meaning of the protein to perform its function. Also, the data from the enzyme analysis can serve in addition, as a discriminator. Also, the data from the enzyme analysis can serve in addition, as a discriminative marker, for the diagnosis of conditions with copper dyshomeostasis.

The clinical applications of the methods approved by Dr. Ivanova are related to several important aspects:

Investigation of copper in serum in healthy individuals in Bulgaria. The dependences of serum copper on geographic location, smoking, alcohol use, and degree of physical activity were established.

In clinically healthy individuals, data are presented to characterize the copper status with oxidase and specific activity of ceruloplasmin. A comparison was made of the measured limits of a reference area for serum copper in 1987 and in 2015. The need for their updating has been proven.

Research on the relationship between serum copper and neurodegenerative diseases - Alzheimer's disease - is significant. After measuring the serum copper levels of individuals from certain regions in Bulgaria, the number of hospitalized individuals with Alzheimer's disease for these regions was also found. A proportional relationship between them has been established. Investigations of total and "free" copper in cerebrospinal fluid are not yet standardized. But non-ceruloplasmin-bound copper is a new candidate marker for early risk stratification for neurodegenerative diseases.

Dr. Ivanova sheds new insight on the so-called copper balance by examining the "activity" of ceruloplasmin. The internal distribution of copper fractions in the sense of total serum copper is important. The representativeness of "free" copper, which is not in complex with ceruloplasmin, is the one to which the toxic effects are due, since copper ions have a high reactivity and easily involve into oxidation-reduction processes. Nuances in the knowledge of trace elements in normality and pathology are related to the balanced redistribution in the cell and the dynamic equilibrium between the protein-bound fractions and those of the labile pool. Modern terminology defines copper imbalance as the toxic accumulation of non-ceruloplasmin-bound copper. Dr. Ivanova conducted the first study comparing the oxidase activity of ceruloplasmin in healthy subjects and in patients with Wilson's disease. Lower levels of activity were found in patients. This is due to the reduced resistance of the protein in the absence of copper in its structure. Ceruloplasmin synthesis is not impaired (as in aceruloplasminemia). The half-life of the molecule is reduced. Accordingly, the activity of the enzyme is decreased, as well as the ability of the molecule to deliver copper to the place of use. Copper ions are part of the active center of a number of metalloenzymes important for many processes: neurotransmission, erythropoiesis, pigmentation, oxidative processes, glucose tolerance and etc. Therefore, the clinical picture of Wilson's disease is very diverse. As a diagnostic criterion in patients with newly diagnosed Wilson's disease, the oxidase activity of ceruloplasmin has a higher diagnostic specificity compared to its concentration - (100% vs. 78.8%).

#### **Conditions with copper dyshomeostasis (G7.2, G7.5, G7.6, G7.7, G8.3, G8.7, G9.1):**

Conditions of copper dyshomeostasis appear to be not so rare. These conditions are classified as "deficiency" or "accumulation" of copper in the body. The use of this classification has certain reservations, since there is a redistribution of copper ions at the cellular and systemic level. In her review, Dr. Ivanova presents rare conditions of copper imbalance reported in a small number of patients worldwide. Modern terminology is introduced, which is closer to the pathogenetic mechanisms of disorders in copper exchange, namely "**ATPase diseases**." For example, **ATP7A - Menkes ATPase; ATP7B – Wilson ATPase**. Based on genetic evidence for small genetic variants or mutations in the ATP7B gene, an analogy has been made between the rare condition Wilson's disease and the socially significant form of dementia Alzheimer's disease. Dr. Ivanova presents current data for reference areas according to gender and age for copper, ceruloplasmin and cupriuresis. New markers for the characterization of copper exchange are indicated, which may also be used in pathologies related to inflammatory processes. These are: determination of the free fraction of copper; ceruloplasmin activity;



copper/ceruloplasmin ratio; copper/zinc ratio; the ratio ceruloplasmin concentration/ceruloplasmin enzyme activity, etc.

**Reference limits for serum copper and determination of biological variations in healthy Bulgarians (A.1, D7.1, D7.9, D8.8):**

Reference limits of serum copper and characterization of biological variations in healthy Bulgarians is an important scientific contribution of Dr. Ivanova's work, which was developed in her dissertation and related publications. In Bulgaria, there was an urgent need to update these studies.

**Study of stress markers; new markers in liver pathology (NAFLD) and in kidney diseases; micronutrients and vitamins (G7.3, G7.4, G7.8, G7.10, G7.12, G8.2, G8.4, G8.5):**

Broad scientific interests are represented in the study of markers of stress, markers of liver pathology - NAFLD, of kidney disease, micronutrients and vitamins.

A study of salivary cortisol levels in employees working 12-hour shifts in casinos showed no deviations in hormone dynamics compared to known circadian dynamics. But statistically significant are the higher levels of cortisol in women compared to men. In a study of 39 Bulgarian welders with the same material and method, morning values were found to be higher than the reference range. Research is useful for stress prevention and raising the awareness of workers in order to sensitize them to problems in the occupational environment.

In Bulgarian patients with non-alcoholic hepatic steatosis and obesity, with and without prediabetes, the levels of PNPLA3 (patatin-like phospholipase domain-containing protein 3) were investigated. Carriers of the PNPLA3 1148M variant were found to have a 9.6-fold higher risk of glucose disturbances compared to the wild-type genotype (at corresponding confidence intervals). These studies prove for the first time in our country that PNPLA3 1148M is associated with an increased risk of prediabetes, metabolic syndrome and insulin resistance in patients with nonalcoholic steatosis of the liver and obesity. The rs738409G variant is associated with higher blood sugar, dyslipidemia and higher liver enzyme values. For early diagnosis of kidney damage, Dr. Ivanova study serum uromodulin levels. The accumulated data support the use of serum uromodulin as a potential marker for the diagnosis and early assessment of the progression of chronic kidney disease.

In patients with psoriatic arthritis, Dr. Ivanova and her colleagues conduct important studies of: serum levels of vascular endothelial growth factor (VEGF), cartilage oligomeric protein (COMP) and matrix metalloproteinase 3 (MMP3), comparing them with CRP levels

and clinical status of the patient. The highest correlation with the degree of the aggravated clinical picture was shown by the levels of VEGF.

A new group of patients and healthy pregnant women were examined for vitamin D levels - those with autoimmune diseases. There are no specific guidelines for pregnant women regarding vitamin D supplementation. The data from this study confirm the known facts about impaired vitamin D status in women of reproductive age and during pregnancy. However, 25-OH-D3 levels in pregnant women were found to be statistically significantly higher than non-pregnant women levels. Supplementation during normal pregnancy during periods of limited sunshine will allow to get out of the deficiency zone. It can be assumed that pregnancy is associated with specific physiological mechanisms aimed not only at overcoming the neonatal deficiency, but also at ensuring higher levels of 25-OH-D3 in the maternal organism. A significant deficiency of 25-OH-D3 was found in patients with autoimmune diseases. Replacement therapy with vitamin D is recommended, as the immunomodulatory effect of the vitamin will be a useful therapeutic tool in these patients.

### 3. Teaching and learning activity

Dr. Ivanova's educational and teaching activities are related to the training of medical students in the "Clinical laboratory" discipline and the "Clinical chemistry" discipline - for pharmacy students at the Faculty of medicine of Sofia University; with the training of students in the "Clinical chemistry" course for the specialty Pharmacy in English at the Faculty of Chemistry and Pharmacy of Sofia University; of students in the "Chemistry" department of the Faculty of Science and Mathematics at the Southwest University "Neofit Rilski"-Blagoevgrad. Academic commitment is related to teaching in both Bulgarian and English. For the period 2016-2022, Dr. Ivanova has a total of 1647 teaching hours, of which 66 hours are lectures and 1581 hours are exercises. The data indicate that Dr. Ivanova is an experienced and established academic teacher in the disciplines "Clinical laboratory" and "Clinical chemistry".

### 4. Diagnostic and therapeutic activity.

Dr. Ivanova is the Head of the Clinical Laboratory at UH "St. Ivan Rilski" - Sofia - a prestigious hospital with a prestigious laboratory - the highest Third level of competence according to the Clinical Laboratory Standard in Bulgaria. As such, she is responsible for the organization of more than 20 specialist staff and the realization of over 510,000 studies per year. New equipment and new methodologies are implemented and tested annually in the laboratory. As an experienced specialist in the medical specialty "Clinical laboratory", she carries out consulting work in other metropolitan laboratories and hospitals.

## 5. Short-term specializations and qualifying courses. Awards.

- a) In 2022, Dr. Ivanova was nominated and awarded the Balkan Medical Scientists Award for Innovation.
- b) She is a member of the "Federation of Laboratory Medicine" with certificate No. 87093
- c) She is certified by the EFLM (European Federation of Clinical Chemistry and Laboratory Medicine) as a European specialist (EuSpLM) in laboratory medicine with registration No 175.
- d) In 2017 Dr. Ivanova was awarded second prize for a poster presentation at the National Clinical Laboratory Conference.
- e) Dr. Ivanova presents certificates for a number of postgraduate studies at seminars and conferences.

## 6. Personal impressions:

My brief meetings with Dr. Irena Ivanova have been fruitful and pleasant colleague conversation.

## **Conclusion**

Dr. Irena Ivanova is an established professional, scientist and academic lecturer. Her scientific production and teaching activity, as well as her professional development, indicate quantitative sufficiency and high quality professional performance.

I strongly recommend the respected scientific jury to award the academic position of "Associate professor" (0.25% full-time position) to Irena Dimitrova Ivanova, MD, PhD.

Date: 07.12. 2022  
Sofia

Signature:.....