

STANDPOINT

on the materials for participation in a competition for the academic position "Associate Professor" in a professional field 4.3. Biological Sciences (Microbiology) for the needs of the Department of Infectious Microbiology, Cytotoxicity and Signal Transduction Laboratory at the Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, published in the State Gazette, issue no. 47, p. 56, of 22 May 2020.

Candidate for participation in the competition: Chief Assistant Professor Maya Margaritova Zaharieva, PhD.

Standpoint prepared by: prof. Teodora Petrova Popova, DVM, DSc, University of Forestry, city of Sofia, field of higher education "Agrarian sciences and veterinary medicine", professional area 6.4. "Veterinary medicine", scientific specialty "Epizootology, infectious diseases and prevention of contagious diseases in animals" (Microbiopogy), appointed as member of the Scientific jury by Order No. I: 83/01.07.2020 of the Director of the Institute of Microbiology - BAS.

1. Brief biographical data about the candidate.

Maya Margaritova Zaharieva graduated from Goethe High School in German in Burgas, and in the period 01.10.1993 - 30.06.1995 she studied German philology at Sofia University "St. Kliment Ohridski". From 1996 to 2002 she worked as a German translator in Burgas. In 1996 she continued her studies at the Faculty of Pharmacy (FP) at MU-Sofia, which she graduated in 2001 with a Master's degree in Pharmacy. In the same year she specialized at the German Cancer Research Centre (GCRC) in Heidelberg, Toxicology and Chemotherapy Unit (TCU) under the Erasmus/Socrates program with a scholarship for graduate students. She worked part-time as a master pharmacist in pharmacies in Sofia (2002 - 2012). In 2002-2004 she held the same position at Sitonet Sofia Ltd., University Hospital "Queen Joanna". During the period 14.03.2003 - 30.09.2006 she was a full-time doctoral student at the Department of Pharmacology, Pharmacotherapy and Toxicology at the Faculty of Pharmacy, Medical University - Sofia. From 01.10.2004 to 31.12.2005 with DAAD scholarship for doctoral students Maya Zaharieva has specialized in GCRC in Heidelberg, TCU, where she has developed the experimental part for her dissertation, and has participated in courses for professional development. After successfully defending her dissertation, she received the educational and scientific degree "Doctor of Philosophy" in the scientific specialty "Pharmacology (including pharmacokinetics and chemotherapy)", awarded by the Higher Attestation Commission on 09.06.2008. On November 1, 2007 Dr. M. Zaharieva entered FP, MU - Sofia as a research associate on a project in the field of molecular pharmacology and experimental chemotherapy. From November 1, 2008 to October 31, 2010 she was the head of a research project under the program for young scientists of the Alexander von Humboldt Foundation at the GCRC in the field of toxicology and chemotherapy. From 01.06.2011 to 31.05.2012 Dr. Zaharieva has worked as a research associate in a project under the reintegration program of the foundation "Al. von Humboldt"- Center of Excellence for Translational Research in Hematology. In 2012 she has been a university lecturer (assistant) in pharmacology at the Faculty of Medicine (FM) at Sofia University "Kliment Ohridski", Lozenets Hospital and has participated in experimental activities on research projects. From 12.07.2012 to 04.07.2014 Dr. M. Zaharieva has worked as an assistant professor in microbiology at the Institute of Microbiology (IMic) "Stefan Angelov", BAS. From 04.07.2014 until now she is a senior assistant professor chief assistant professor in microbiology at the same institute, where she is engaged in experimental work on research

projects, preparation of scientific publications, dissemination of scientific results, training of graduates and doctoral students and preparation of textbooks. During her work activity she has constantly improved her qualification, having participated in a number of courses and specializations at home and abroad and has mastered topical laboratory methods. In addition, Dr. Maya Zaharieva is fluent in German and English, also uses Russian and French. She has significant experience in working with modern specialized laboratory equipment and methods, as well as computer skills and competencies at a high level. She has excellent communication skills and experience in teamwork, acquired during her work, teaching and research.

2. Compliance of the submitted documents and materials by the candidate to the requirements according to the Rules for development of academic staff at IMic-BAS.

Ch. assistant professor Dr. Maya Zaharieva has presented all the documents necessary for participation in the competition for the academic position (AP) "Associate Professor". They have been prepared according to the requirements of the professional field and the Regulations for DAS in IMic-BAS. This has been also established by the Commission appointed for this purpose by the Director of IMic-BAS.

3. Assessment of the candidate's scientific, applied scientific and publication work.

Ch. Assistant Professor Dr. M. Zaharieva has a total work experience in the specialty so far 19 years and 4 months. For participation in the competition for associate professor she has presented a total of 28 scientific papers, which do not repeat those for the acquisition of "PhD" degree (the latter include a dissertation, abstract and 4 publications in English).

The total number of points obtained by the groups of indicators is 1030 with minimum national requirement of 400 points.

- By indicator 1 (group **A**) ch. assist. prof. Dr. Zaharieva has 50 points out of 50 required - for dissertation for the acquisition of "PhD" degree on "Study on the pharmacodynamic effects of erufosine in tumor-transformed and normal hematopoietic cells", defended in 2008.

- By indicator 2 (group **B**) - 0 points out of 0 required.

- By the indicators from gr. **B** - 211 points out of 100 required, obtained from 11 scientific publications in editions, referenced and indexed in world-famous databases with scientific information.

- By the indicators from gr. **Г** - 299 points out of 200 required, of which: ♦ Articles and reports published in scientific journals, referenced and indexed in world renowned databases with scientific information - 14 pcs. - 254 points. ♦ Published chapters from books or collective monographs - 3 pcs. - 45 points.

- By the indicators from gr. **Д**, the candidate has received 470 points out of 50 required by 235 citations (x 2 points per citation) in scientific journals, referenced and indexed in world renowned databases with scientific information (Web of Science, Scopus).

- By the other indicators - 0 points out of 0 required.

The research assets of Dr. Maya Zaharieva significantly exceed the additional criteria for the development of academic staff at IMic-BAS. The number of her publications with IF is 27 (at required 20), of which 21 are articles with JCR-IF, 3 - book chapters and 3 - articles in collections of international forums. In 7 of them (at minimum of 5 required) she is the lead author, including 5 articles with JCR-IF, the first place in one of them being shared with another co-author, in 1 chapter of a book and in 1 article in a collection of congress. Dr. Zaharieva has 235 presented citations (Scopus) until 17.07.2020 at required 100 pcs. Her JCR-IF is 45,724 at needed minimum of 20. Her H-factor is 9 (Scopus) at minimum of 5 and she has 14 participations in projects when 3 are required.

The total number of points received by Dr. Zaharieva on all indicators (1030) more than twice exceeds the minimum needed to meet the minimum national and additional requirements

in the professional direction, defined by the Rules of the DAS of IMic-BAS for occupation of the academic position "Associate Professor" In the field of higher education 4. Natural sciences, mathematics and informatics. These data are an indicator of a high level of professional qualification and significant results achieved in the scientific field.

3.1. Participation in scientific, applied scientific and educational projects.

Dr. M. Zaharieva has participated in the performance of 14 research projects implemented in the period 2014 - 2019, at three of which she has been the head. This asset is a testament to her significant experience and skills as a researcher and leader of creative teams.

3.2. Characteristics of the published scientific results.

Of the 28 scientific papers submitted for participation in the competition, Ch. assist. prof. Dr. Maya Zaharieva is the first author of 5 publications with JCR-IF, one chapter of a book, 1 article in a collection of international congresses, as well as 1 publication in a journal with SJR. The candidate is a co-author in 16 publications with JCR-IF, in 2 book chapters and in 2 articles in proceedings of international congresses. For the period 2014 ÷ 2019 data are presented for participation of Dr. M. Zaharieva in 36 scientific conferences, 10 of which abroad. This activity in the research work is impressive and deserves high evaluation.

3.3. Reflection of the candidate's scientific work in literature (citability).

Data are presented about 279 citations of 23 publications with the participation of M. Zaharieva for 2005 - 2020. For the period after the acquisition of "PhD" degree (after 2008) so far they are 263. In the attached reference for compliance with the minimum requirements of the Law for development of the academic staff in the Republic of Bulgaria (LDASRB) and in the reference for the additional requirements of IMic-BAS is presented the number of citations, taken out only from the Scopus database, which can be seen online. Until 17.07.2020 they are 235 (without auto-quotes of all co-authors). The other citations in the presented list are from the Google Scholar database, but they are not taken into account when calculating the points. This is a very high result, testifying to the significant impact of the candidate's scientific output in world literature and proof of her significant contribution to the development of science in the fields in which she works.

3.4. Contributions in the candidate's works (scientific, applied scientific, applied).

The numerous original scientific contributions of the candidate from the articles, which are equivalent to the Habilitation thesis, are of valuable fundamental and applied nature in existentially important fields of knowledge such as oncology, pharmacology, toxicology, infectious microbiology and bacteriology in general. They can be grouped into the following four scientific fields: ♦ Antineoplastic activity, cytotoxicity and signal transduction of extracts, biologically active substances (BAS) and synthetic compounds in malignantly transformed cell lines. ♦ Antimicrobial effect of plant extracts, BAS and human neutrophil peptides (defensins). ♦ Toxicological analysis of substances and extracts with antineoplastic and antimicrobial activities *in vitro* and *in vivo*. ♦ Modeling with MAPLE mathematical software of malignant cell proliferation and metabolic activity in bacteria.

Of particular importance in the first and second areas are the contributions related to: • Antineoplastic effect and mechanism of action of the alkylphosphocholine erufosine in leukemic, lymphoma and tumor cell lines. • Antimicrobial action of new strains of microalgae, as well as alpha-defensin 2 in pharmacopoeial buffers with different pH. In the third area, contributions can be grouped as follows: • Toxicity studies of ethyl acetate extract of *Geum urbanum* L. on intestinal epithelium and Peyer's patches. • Determination of the cytotoxic effect of GOTCAB saponins, as well as of eye prostheses with antimicrobial activity. • *In vitro* toxicological evaluation of platinum complexes with acetate ligands. Contributions from the fourth area are related to modeling the processes of inhibition of proliferation of human tumor cell lines and metabolic activity in bacteria with mathematical software MAPLE.

An important asset is also the leading role of the candidate in the creation and development of the Laboratory "Cytotoxicity and signal transduction" for the needs of the Department of Infectious Microbiology at IMic-BAS. Hence come a number of practical contributions related to the expansion of the infrastructure of this department and the introduction of new scientific directions. Particularly important in this aspect are the contributions related to: • Introduction of routine protocols for storage, cultivation and measurement of biochemical parameters in cell cultures. • Creating a collection of normal and tumor cell lines. • Introduction of the principles of 3R when working with experimental animals.

Guidelines for the future development of the laboratory are also outlined, which are related to the listed scientific areas and are based on collaborative projects approved for funding. They are the following: • Testing of the antineoplastic and antimicrobial activity and elucidation of the mechanism of action of new medicinal candidates of natural and synthetic origin. • Characterization of the toxicological profile of natural and synthetic substances with antimicrobial activity. • *In vitro* study of interactions between virulence factors in food pathogens and host cells. • *In vitro* study of interactivity between bacterial pathogens/toxins and host cells associated with the carcinogenesis of certain neoplasms.

These contributions are a valuable input to science and an indicator of significant activity in research with prospects for future development.

4. Assessment of the candidate's educational teaching work.

During her work at FF, MU - Sofia as a research associate on a project in 2007 - 2008, Dr. M. Zaharieva has participated not only in conducting *in vitro* experiments, but also in training graduates and doctoral students. In 2011 as an assistant professor of pharmacology at the Faculty of Medicine at Sofia University "Kl. Ohridski", Lozenets Hospital, she has taught pharmacology exercises for students majoring in Medicine, Nurse and Occupational Therapy and Rehabilitation. In the academic year 2011/2012, Assistant Professor Dr. M. Zaharieva has conducted 327 academic hours in FM at Sofia University "St. Kliment Ohridski". Of these, 315 hours are exercises in the discipline "Pharmacology" with students at Sofia University, 270 hours of which are in the specialty "Medicine" (Master's degree) and 45 hours - "Nurse" and "Medical Rehabilitation and Occupational Therapy" (Bachelor's degree). She also has 12 hours of extracurricular activities, as in the same school year she has examined 40 students in the same specialties. She has been a thesis co-supervisor for three students, two of whom were foreign students in the English course. She has involved in the preparation of two chapters (№5 and 8) of the textbook "Guide to Pharmacotherapy. Ch. editor S. Konstantinov. Ed. Softtrade, Sofia, 2011. ISBN 978-954-334-1 19-1".

These data clearly show that Ch. assist. prof. Dr. Zaharieva has teaching experience in conducting practical classes and exams in her discipline with students from different specialties, as well as experience in training of graduates and preparing textbooks.

5. Other activities in relation to the scientific specialty.

The candidate Ch. assist. prof. Dr. M. Zaharieva has a significant contribution to the construction and equipment of specialized laboratories. Already in 2002 - 2004 in Sitonet Sofia Ltd., University Hospital "Queen Joanna" she has such a participation in a laboratory for the production of transplants of human hepatocytes, as well as in the development of new methods for isolation and freezing of human hepatocytes and the introduction of SOP-system for production and quality control according to the requirements of good production and laboratory practice. Later, her contribution has been significant in the creation and development of the Laboratory "Cytotoxicity and Signal Transduction" at IMic-BAS. She also

has an important role in receiving in this institute of specialized equipment, a donation from the foundation "Al. von Humboldt".

Maya Zaharieva has participated in educational courses essential for her work with modern methods and equipment, e.g. in the production of viral particles and viral transfection of eukaryotic cells, for work with a flow cytometer, in immunology and immunological methods, in model cell systems, in transmission electron microscopy, for NGS (next generation sequencing) with Illumina devices, for autoclaves and sterilization of equipment and etc. She has passed a number of trainings and specializations for raising the qualification in the scientific field, namely: • Course in cellular model systems, CNR -Tor Vergata in Rome, Italy (05.06.2011 - 10.06.2011). • Specialization in quantitative PCR, based on TaqMan probes at the Institute of Agriculture and Fisheries, Merelbeke, Belgium (10.10.2012 - 10.12.2012). • Identification of pathogens in food samples with quantitative PCR based on TaqMan probes (real-time PCR, absolute quantification). • Specialization in loop-mediated DNA amplification for identification of pathogens in food matrices at the Institute of Agriculture and Fish Farming in Merelbeke, Belgium (18.05.2015 - 16.07.2015). ▪ Determination of chemosensitivity and cytotoxicity of compounds of synthetic and natural origin, incl. mechanism of action *in vitro* and *in vivo*. ▪ Identification of pathogens in food samples with loop-mediated DNA amplification (18.05.2015 - 16.07.2015).

As a result of these trainings and her long-term research work, she has mastered important modern laboratory methods: ▫ Isolation and cultivation of primary cultures, cell lines and bacteria. ▫ Assessment of cell viability with MTT test, CellTiterGlo; CellTiterBlue; ATP and other colorimetric, luminescent and fluorimetric methods. ▫ Assessment of clonogenicity with CFU test. ▫ Migration test. ▫ Determination of minimum inhibitory and minimum bactericidal concentrations by EUCAST methods and international standards. ▫ Electrophoresis (of proteins and DNA). ▫ Determination of expression and enzymatic activity of proteins. ▫ Isolation of lipid shelves. ▫ Electroporation and transfection of nucleic acids into prokaryotic and eukaryotic cells. ▫ Working with phase-contrast and fluorescence microscope. ▫ FACS. ▫ Recombinant DNA technique. ▫ Isolation of eukaryotic and bacterial DNA, RNA and proteins. ▫ Loop-mediated DNA amplification. ▫ RT-PCR, qPCR, ddPCR. ▫ Investigation of antibacterial activity with microtitration and agar-gel-diffusion methods, determination of anti-biofilm activity according to Stepanovich's protocol. ▫ Determination of reduction capacity of cell models with Elman's reagent. ▫ Various ELISA and colorimetric tests for biochemical processes.

Part of the candidate's activity has been evaluated with dignity with the following awards and prizes: ▫ Grant for young scientists, funded by the foundation "Al. von Humboldt" at the German Cancer Research Centre in Heidelberg, Germany (October 1, 2008 - October 31, 2010). ▫ Participation in an article that won second place in the Top 25 for 1-4 months of 2012 in the scientific journal Cancerletters, Elsevier. ▫ Won a grant for equipment for a cell cultures laboratory from the foundation "Al. von Humboldt" (October 6, 2014). ▫ Award of the pharmaceutical company TEVA for the best poster of the "2nd International Conference on Natural Products Utilization", Plovdiv, Bulgaria, 14-17.10.2015. ▫ Best poster presentation of "Pre-Conference Workshop on Food Borne Pathogens and Food Safety", Sofia, Bulgaria, 26-27.05.2016.

Dr. Maya Zaharieva is a member of the Union of Humboldtians in Bulgaria, of the Union of Pharmacists in Bulgaria, and since April 25, 2013 - of the Union of Scientists in Bulgaria.

These numerous activities and awards further illustrate the multifaceted development of the candidate as an excellently prepared and promising researcher in her scientific field.

6. Assessment of the candidate's personal contribution.

Ch. assist. prof. Dr. Maya Zaharieva is the **first** author of a total of **8** publications from the 28 scientific papers submitted for participation in the competition. She is the **second** author of **6** of the publications and the **third** of **5** of them. These data prove her leading role in most of the presented works and clearly outline her significant personal contribution to the scientific production.

7. Critical remarks and recommendations.

I do not have such.

8. Personal impressions.

I have no personal contacts with Dr. Zaharieva, but the presented documentation perfectly characterizes her as a remarkable scientist. I am impressed and highly appreciate its precision, high competence and activity as a researcher, which is evident from the scientific production with high JCR-IF, the activity of implementation and management of scientific projects, valuable scientific contributions and those in the creation and equipment of laboratories, numerous citations and her active participation in scientific forums at home and abroad.

9. Conclusion.

Based on the information presented above, I confidently give my positive vote, allowing myself to suggest to the esteemed members of the Scientific Jury also to vote positively and the candidate Chief assistant professor Dr. Maya Margaritova Zaharieva, PhD to take the academic position of "Associate Professor" in a professional direction 4.3. Biological Sciences (Microbiology) at the Department of Infectious Microbiology, Laboratory "Cytotoxicity and Signal Transduction" at the Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences.

02 August 2020
City of Sofia

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