To the Chairman of the Scientific Jury
Director's order No. I-84 / 01.07.2020
The Stephan Angeloff Institute
of Microbiology (SAIM), BAS

REVIEW

Reviewer: Prof. Todor Veselov Kantardjiev, MD, PhD, DSc, M.H.M
Institution: Director, National Center for Infectious and Parasitic Diseases, Sofia

Regarding: Competition for the occupation of the **academic position"Associated Professor"**, Scientific field 4.3. Biological Sciences, scientific specialty "Microbiology" announced in the State Gazette issue 47/22.05.2020 for the needs of Department of Infectious microbiology, Laboratory of molecular biology of mycobacteria, The Stephan Angeloff Institute of Microbiology (SAIM), BAS **with the only one candidate Assistant Professor Violeta Valcheva Ruseva, PhD**

I declare that I do not have any common publications or conflict of interests according to the Law of the Development of the Academic Compositions in the Republic of Bulgaria with the only candidate for the Academic Position "Associated Professor"

1. Received materials for review

The candidate Assistant Professor Violeta Valcheva Ruseva, PhD was admitted to participate in the competition by the Commission by Director's order No. I-84/01.07.2020. The presented documentation for participation in the competition fully complies with the requirements of the Law on Academic Growth in the Republic of Bulgaria, the Regulations for its application and the Regulations for its application in the SAIM, BAS. From the presented documents it is evident that the professional qualification of Assist. Prof. Violeta Ruseva, PhD, corresponds to the indicated habilitation in the specialty.

2. Scientific and career growth of the candidate

Assistant Professor Violeta Valcheva Ruseva began her professional career as a biologist in the Department of Pathogenic Bacteria, SAIM, BAS in 2004 and then she obtained a master's degree at Sofia University "St. Kliment Ohridski" specialty "Microbiology and Microbiological Control" in 2005. As soon as she finished her higher education, she started her Doctoral thesis in the same department of the institute and in January 2009 she got the educational and scientific degree "PhD" after defending a dissertation on the Molecular genetic characterization of *Mycobacterium tuberculosis* strains, isolated from different regions of Bulgaria". In the same year she was appointed as a Assistant professor in the Department of Infectious Microbiology. During the doctoral's thesis period till now the candidate participates intensively in various national and international projects, attends courses and acquires extensive experience and high qualification in many international institutes (including Pasteur Network Institutes in Europe and Asia) in the field of molecular epidemiology research on mycobacteria and bioinformatics.

All acquired skills are directly applied in the scientific work of the candidate, achieving visible results (increasing the number of projects, publications, creating new national and international collaborations, awards of the Union of Scientists in Bulgaria). As a leader of three national projects of National Science Foundation and one of the BAS, as well as an active participant in international projects (NATO, FP7, Russian Science Foundation) and international networks (International Research consortium on tuberculosis and other mycobacterial diseases (FATE) and European Cooperation in Science and Technology (COST)) gives her a good opportunity for current and future collaborations with Pasteur Network institutes and other national and international organizations. The ascending career development of Dr. Violeta Ruseva evidences of persistent and systematic upgrading of specialized knowledge in the field of tuberculosis control in humans and animals. Gaining significant experience in project management, participation in various courses, most of which abroad as well as specializations in several international institutes of the Pasteur network ensure the implementation of in-depth research on selected topics, acquisition of knowledge and skills in the long run and career growth. Along with the described activities, the candidate also shows organizational qualities. She is responsible for "public relations" at the Institute of Microbiology, respectively at the network of Pasteur Institutes for Bulgaria where she has the task to establish and maintain continuous bilateral communication on the one hand with other institutes of BAS and Central Administration and on the other with the institutes of the Pasteur network. This position gives her the opportunity to constantly communicate with scientists and media from Bulgaria and abroad, which has great opportunities for the transfer of knowledge and information among a wide sociality in and outside our country. Dr. Violeta Ruseva, has developed as a scientist entirely at the Institute of

Microbiology, where she actively participated in scientific life and rightly won twice the award for High Scientific Achievement of a young researcher from the Union of Scientists in Bulgaria, Section "Microbiology" in 2009 and 2011.

3. Research activity

3.1. Scientometric indicators according to the Law of the Development of the Academic Compositions in the Republic of Bulgaria and its regulations

Group of indicators	Contents / Indicators	Associate Professor /Points	**Documents applied by the Candidate**
A	Indicator 1 Dissertation for the educational and scientific degree "PhD"	50	**50**
B	Indicator 4 Habilitation work - scientific publications in peer-reviewed journals indexed in world-famous databases of scientific information (Web of Science and Scopus)	100	**130**
C	Sum of indicators from 5 to 10 Scientific publications in peer-reviewed journals indexed in world-famous databases of scientific information (Web of Science and Scopus), Published chapter of books	200	**237**
D	Sum of points in indicator 11 Citations in scientific journals, monographs, collective volumes and patents, referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus)	50	**482**

Dr. Violeta Ruseva entirely covers the criteria of the Law of the Development of the Academic Compositions in the Republic of Bulgaria and the Regulations for its application. According to indicator B, the candidate presents 6 publications with a total number of points 130 with a minimum requirement of 100 points. The total number of points on indicator "C" is 237

for the required 200, for 10 scientific publications in peer-reviewed journals indexed in world-famous databases of scientific information (Web of Science and Scopus), as well as 2 published book chapters in international books. There are a total of 241 citations, mostly by foreign authors. Total number of pointsfor group "D" is 482 with a minimum of 50 points.

3.2. Scientometric indicators according to Appendix 2 of the Regulations for academic positions at the Stephan Angeloff Institute of Microbiology, BAS

Academic position "Associate Professor"	Number of publications in journals with IF, publications of international forums, published in full text	Citations	IF	h-index	Additional requirements
Annex 2 of the SAIM Rules for the Associate Professor	20 (excluding those included for "PhD") in 5 of them 1st author	100	20	5	Participation in 3 projects
Applied by the candidate	**24 – in 7 of them 1st author**	**241**	**40,295**	**13 (Scopus)**	**14**

A total of 24 scientific publications were submitted for review, of which 2 chapters from international books, 15 publications in international journals with impact factor (IF), 1 publication in an international journal without IF, 1 publication in a national journal with IF and 4 publications in national journals without IF. In 7 of the publications the candidate is the first author and participates with an author's reference with 241 citations, 44 abstracts for participation in national and international forums, of which over half (30) are abroad and 14 participations in national and international projects. The total IF of the participating publications is 40,295 and the h-index for the entire scientific career of the candidate is 13. The candidate's works are highly evaluated by the scientific community abroad, as seen by the large number of international citations, the high h-index and 15 of the articles submitted for review are in international journals with IF. The presented impressive scientific production shows an ascending scientific development and an established scientist. Dr. Violeta Ruseva is a co-author

in various Bulgarian and international groups, which shows that she is a potential partner for joint research.

4. Main scientific directions and contributions

The research activity and the scientific contributions in which Assist. Prof. Violeta Valcheva Ruseva is a leading researcher are grouped thematically in several main areas:

4.1. Identification and molecular epidemiology of tuberculosis in animals

The introduction of molecular genetic methods in the diagnosis of bovine tuberculosis makes it possible to establish the genetic diversity of *Mycobacterium bovis* strains circulating among the cattle population in the affected regions of Bulgaria. The in-depth study of genetic variations supports the development of molecular epizootology in veterinary medicine, to monitor the dynamics of the epidemiological and epizootological process, transmission, evolution and phylogenetics of *M. bovis,* in order to carry out effective epidemiological surveillance of tuberculosis in the country, risk assessment of the emergence of new tuberculosis outbreaks.

In this direction, Dr. Violeta Valcheva Ruseva has made an outstanding contribution to the first molecular genetic study for Bulgaria in the field of animal husbandry, on the genetic diversity of *Mycobacterium bovis* strains circulating in Bulgaria, information has been added to the global database of Bulgarian strains and established for the first time the circulation of *Mycobacterium caprae* strains in Bulgaria.

4.2. Prevalence of paratuberculosis in wild animals in Bulgaria

Paratuberculosis is a disease of large and small domestic and wild ruminants and is important for international trade because it causes serious economic losses to the affected farms due to reduced milk production and meat production.

In this direction, Dr. Violeta Ruseva shows that for the first time in 40 years an in-depth study was conducted for the presence of paratuberculosis in deer and mouflon freely living in hunting farms in Bulgaria. The characteristic of pathoanatomical changes established for paratuberculosis were confirmed by histological, microbiological and molecular genetic studies. The existence of paratuberculosis in wild animals in Bulgaria and the need for a deeper study of the spread of the disease in both wild and domestic ruminants in their habitats need to be proven.

4.3. Molecular epidemiology, drug resistance, phylogenetics and evolution of *Mycobacterium tuberculosis* in Bulgaria and around the world

The fight against tuberculosis is one of the most significant societal challenges in the world. Tracking the population dynamics of *M. tuberculosis*, the diversity of genotypes and their distribution occupy an important place in modern epidemiological studies of tuberculosis in Bulgaria and around the world. Accurate identification of the sources of infection and tracing the routes of its spread contribute to the epidemiological control and tuberculosis prevention strategies.

This direction is one of the most long-term studies and interests of the candidate, dating since 2006, where she proves that the widespread of *M. tuberculosis* spoligotype ST125 in Bulgaria is philogeographically specific for our country. An original contribution is that the local circulation of specific clones of *M. tuberculosis* is an important factor in molecular epidemiological studies of tuberculosis in Bulgaria and that the acquisition of drug resistance and the spread of resistant strains in our country is not related to the population structure of *M .tuberculosis*. It has also shown that the migration of the human population is a major factor shaping the phylogeography of *M. tuberculosis* over long periods of time.

4.4. Establishment of L-forms in mycobacteria and staphylococci

It has been suggested that mycobacteria may persist in host organisms as abnormal morphological forms (L-forms) in an inactive latent state. Mycobacterial L-forms are defined as difficult to isolate, cultivate and identify in clinical materials by standard microbiological methods. As contributions in this direction, Dr. Violeta Ruseva gives the application of the molecular genetic method (spoligotyping) for identification and typing of L-forms of *Mycobacterium tuberculosis* strains and unique ability of L-forms to grow faster than classical tuberculosis bacilli and their importance for the persistence and latent tuberculosis.

The incidence of *Staphylococcus aureus* infections have stimulated the efforts of many researchers to study the detailed mechanisms in the pathogenesis and epidemiology of these infections. The phenomenon of "heteroresistance" in MRSA has a significant bearing on the emergence of multidrug resistance. As contribution the candidate gives the conversion to L-form and the presence of methicillin resistance in susceptible (mecA-negative) and heteroresistant (mecA-positive) S. aureus strains.

4.5. Development of new compounds with antitubercular activity

Despite the worldwide efforts to successfully treatment, prevention and control of tuberculosis, the ubiquitous circulation of resistant forms of mycobacterial strains remains a serious health problem. The need to search for new antituberculosis drugs is becoming increasingly urgent. As a significant contribution in this direction, the candidate gives the following:

- More than 110 new various chemical structures derived from natural terpenoids - camphor and fenhon have been synthesized. Some of their biological and pharmacological activities have been demonstrated. Some of these derivatives, structurally close to the clinically used drug ethambutol, show significantly higher antimycobacterial activity compared to the reference strain *M. tuberculosis* H37Rv and are quite promising for further development of new antituberculosis agents.

5. Conclusion

In terms of volume, content and quality of the presented scientific production and the active research and organizational activity, professional and scientific experience of the only candidate in the announced competition for the academic position "Associate Professor", Assist. Prof. Violeta Valcheva Ruseva fullycover the requirements of the Law of the Development of the Academic Compositions in the Republic of Bulgaria and its regulations, the additional requirements for academic positions at the Stephan Angeloff Institute of Microbiology, BAS. She is an established scientist and expert in the field of modern molecular genetic research on tuberculosis.

Summarizing, the significant contributions from her scientific developments, evaluated by the scientific community, as seen by the large number of citations, her active participation in national and international research projects I confidently recommend to the members of the Scientific Jury to support the application of the candidate and to propose to the Scientific Council of the Stephan Angeloff Institute of Microbiology, BAS to choose Assist. Prof. Violeta Valcheva Ruseva as "Associate Professor" in the professional field 4.3. Biological Sciences (Microbiology) for the needs of the Department of Infectious Microbiology, Laboratory "Molecular Biology of Mycobacteria", the Stephan Angeloff Institute of Microbiology, BAS.

25.08.2020 Reviewer:

/ Prof. Todor Kantardjiev, MD, PhD, DSc, M.H.M/