TO THE ATTENTION OF THE MEMBERS OF THE SCIENTIFIC JURY, APPOINTED BY ORDER No. I-45/26.03.2025, ISSUED BY THE DIRECTOR OF THE INSTITUTE OF MICROBIOLOGY ''STEFAN ANGELOV'', BAS, SOFIA

REVIEW

by Prof. Dr. Radka Mladenova Argirova, virologist, Clinical Laboratory, Tokuda University Hospital, Sofia

RE: Participation in a competition for the academic position of "Associate Professor" in the professional field: 4. Natural Sciences, 4.3. Biological Sciences (specialty virology) announced in the State Gazette, issue 11/07.02.2025 for the needs of the Laboratory "Experimental Influenza Chemotherapy ", Department "Virology", Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences

To Lora Simeonova Simeonova – PhD, Chief Assistant Professor, Stefan Angelov Institute of Microbiology, Department of Virology, Laboratory of Experimental Influenza Chemotherapy

RESPECTED MEMBERS OF THE SCIENTIFIC JURY,

The only candidate for the academic position of "associate professor" in the announced competition is Lora Simeonova Simeonova. She was born in 1980. She graduated from the National High School for Ancient Languages and Cultures "Constantine-Cyril the Philosopher", profile "Biology" with a defended thesis on the topic "Viruses" - 1999, as well as 95th secondary school in Sofia with teaching in Russian. She graduated at Sofia University "St. Kliment Ohridski", Bachelor - Molecular Biology - 2003 and Master - Virology - 2005. In 2011, she defended her dissertation titled: "STUDY OF THE COMBINED ACTION OF RIMANTADINE AND OSELTAMIVIR AGAINST INFLUENZA VIRUS A" under the supervision of Acad. Angel Galabov, and received the ONS "Doctor" (Specialty "Virology" code 01.06.13). Currently she is a chief assistant in the laboratory "Experimental Influenza Chemotherapy" at the Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences. She teaches also as an assistant in molecular biology and pharmacy in English /since 2007/ at Sofia University "St. Kliment Ohridski", as well as in biology and chemistry in English /since 2009/ at the Lebanese School in Sofia. She has attended multiple short specializations and specialized courses, including on scientific projects in Italy, France, Hong Kong, South Korea, etc. Her versatile language education - English, Russian, Spanish, as well as long years of research and teaching experience speak of theoretical and practical knowledge not only in the field of influenza infection, but also in the vast field of biology, chemistry, and virology in general.

RELEVANCE OF THE CANDIDATE'S PUBLICATION ACTIVITY

I hardly need to convince anyone of the relevance of Lora Simeonova's research topics. Known and described since antiquity, influenza is infamous for the 1918 pandemic, as well as for several later epidemics caused by the antigenic shift of the influenza virus. The wide distribution of the 18 HAs and 11 NAs in various combinations in practically the entire animal world makes it impossible to eradicate the virus, and its segmented genome is responsible for its rapid variability, high mutation rate, and the difficulty in diagnosis in many cases. However, for now, the specific chemotherapeutic drug inhibitors

used – especially those of NA activity – seem to remain stably active. However, it is known that resistance develops to these inhibitors, which necessitates the search for new active inhibitors and drug combinations. In this sense, all of Lora Simeonova's scientific publishing activity is completely contemporary - both for science and practice.

In current competition, Lora Simeonova presents participates with 20 scientific papers, /separately 1 dissertation paper - 2011/, of which the candidate is the first/corresponding author in 5 out of them.She has totaly 236 points (required minimum of 220), some of the publications are with IF. The total IF of Lora Simeonova's publications is 48.03, and the IF for publications after the ONS "doctor" - 45.1 /required minimum - 20/. It is noteworthy that only 2 out of the 20 publications presented are without IF and these two publications are in Proceedings dedicated to the 70th anniversary of the Institute of Microbiology "Stefan Angelov", BAS. She is also a participant in 9 projects - 1 international and 8 national /in one of the national ones she is the leader, and in another - the principal investigator /with funding from the National Science Foundation, Sofia University, Medical University-Varna and Bulgarian companies. A total of 149 citations were found /required minimum -100/. The number of citations in scientific publications and publications referenced and indexed in the world-renowned scientific information databases Web of Science and Scopus is 50 /100 points /required minimum of 50 points/. In addition, Lora Simeonova declares participation in 28 national and international scientific forums, in 12 of them she had a poster presentation and in 16 - an oral report. Participation in projects, as well as in scientific forums, is closely related to her research topic.

Lora Simeonova has divided herself her scientific research and results into 5 separate sections.

The first section focuses on combined approaches to the prevention and/or treatment of influenza infection. In fact, a strategy is applied to combine an etiological viral inhibitor and substances that modify the biological response in the host. These substances include antioxidants and immunomodulatory drugs with proven mono-effects on oxidative stress. The synergistic properties and therapeutic effect on survival, infectious titer and pulmonary pathology of mice experimentally infected with influenza A/H3N2 strain have been demonstrated with simultaneous administration of the antiviral drugs, oseltamivir and rimantadine, in sub- and optimal doses of both drugs. It has been found that the combination with Vitamin E at sub-therapeutic doses of oseltamivir favorably affects both the severity of the disease and survival. The results obtained indicate that the application of antiviral therapy simultaneously with the increase of antioxidant defense in the host is a good approach to control the infection and reduce its severity.

This approach, developed under different experimental conditions, combinations and doses, is also valid for other respiratory infections in which there is overactivation of immunity and active generation of toxic radicals. The highlighted approach is a major contribution to the scientific research activity of Lora Simeonova. The candidate has presented the work and conclusions of this section in 3 papers, which have been cited a total of 46 times. These studies are directly related to current human health problems, aim to improve the quality of life and the development of human resources, and are in accordance with the National Strategy for the Development of Scientific Research 2017-2030, as well as with the European priorities in the field of health and healthcare.

The next section treats oxidative stress in viral infections /not only influenza and not only in humans/ exploring antioxidant properties of natural and synthetic inhibitors. The antioxidant properties of known anti-influenza substances such as blood geranium extract, ellagic acid, oseltamivir and isoprinosine have been characterized in model systems and epigenetic regulation in the cell has been examined as a possible target for antiviral therapy. This section includes 5 publications, 4 out of them have IF. The 4 articles have been cited 20 times. In this section, the candidate demonstrates work with natural, in addition to synthetic inhibitors. In the following section, lactic acid bacteria products were studied for antiviral and virucidal activity against infection in cell cultures infected with human herpes simplex 1 and Koi herpes virus in carp fish. A significant effect was demonstrated, which was not virus-specific within the taxonomic group. The achieved effect is comparable to the standard for virucidal activity of 70% ethanol, as the main active component of disinfectants. In collaboration with the Pasteur Institute Athens and within the framework of the ELPanvir research consortium, a moderate to strong anti-influenza effect of 20 newly synthesized bisartan derivatives with anti-coronavirus activity were established (unpublished data). There are 7 publications in this section, some of them from 2025 /it is too early for citations/, and the citations are 20. I recommend that these studies continue with the search and application of new synthetic bisartans - inhibitors of human respiratory viruses. For the first time in Bulgaria, the susceptibility of influenza virus strains (H1N1) and (H3N2), isolated in the country in the period 2004-2007, to neuraminidase inhibitors and M2 blockers was determined. Sequencing revealed S31N and V27T mutations in the transmembrane region of the M2 protein, responsible for resistance to M2 blockers in the A/Sofia/1250 (H3N2) strain. I would attribute this serious contribution to conclusions regarding applied science. But the essential thing here is that the candidate is able to perform molecular-genetic and phenotypic characterization of important human viral pathogens - influenza virus and HPV, and this has been done for the first time in the country.

The last section includes studies to reveal the potential of nano-carriers of drug substances through studies on cytotoxicity and antiviral activity in classical cell lines. Low toxicity and excellent virucidal properties of silver nanoparticles against influenza A in cells (MDCK, BJ and A549) were demonstrated. A combination of Poloxamer 407 (P407) and hydroxypropylmethylcellulose (HPMC) hydrosols was proposed as an in situ thermogelling carrier for nasal delivery of chlorhexidine–silver nanoparticle conjugates (SN-CX). The results suggest that the developed system can be considered as a potential formulation of a protective nasal spray against respiratory infections. The potential for encapsulation of multicomponent plant extracts in liposomes as optimized delivery systems and reduction of cellular toxicity was investigated as well as using lower doses to achieve bioavailability inside the host cell. It was found that the liposomes prepared in this way exhibit inhibitory effects against the human seasonal beta-coronavirus OC43 (HCoV-OC43) in vitro. **These are important fundamental contributions in the field of pharmacology, and are of great practical importance. 3 articles have been published in this section, cited 7 times so far.**

In general, I completely agree with the contributions formulated by the author herself. Some of the contributions /N 3/ could be combined with N 2, for example, or contribution N 6 could be attributed to the scientific-applied ones, but I accept the author's considerations.

Upon becoming acquainted in detail with the candidate's research activities, I discover a scientist with a clear goal and a long-standing commitment to achieving the goal. The very fact that Lora Simeonova graduated from secondary school with a thesis on "Viruses" and today reaches the rank of "associate professor" in virology speaks of a dream come true with systematic work, accompanied by continuous enrichment with knowledge in an extremely modern field, and it is evident that she will continue to move forward with increasing inclusion of practical tasks in her work. I highly appreciate the candidate's dedication to the field of influenza, not a single publication she publishes is random or piecemeal, she always works purposefully. Research is performed with reliable and modern virological, analytical and molecular biological up-to-date research methods, with the prospect of being continued in the future. The research was carried out in a team of various specialists, but the personal participation of the candidate is always clearly demonstrable - mainly by her participation in 9 projects with departmental and external funding, which is fully consistent with the interdisciplinary nature of modern science. All the conclusions are convincing. Since in her development Lora Simeonova has inherited and continues to develop the field of antiviral therapy with synthetic and natural substances, it is quite understandable that she has expanded this area of research with an active search for new antivirals and/or new therapeutic combinations thereof in order to improve their antiviral activity and reduce resistance and adverse events during therapy. Therefore, there is continuity in thematic terms, combined with updating the research methods, expanding the scope of the targeted synthetic and natural sources - the object of research, and enriching the previously known conclusions with new contributions from these studies.

The academic position of "associate professor" requires serious teaching work. Lora Simeonova's teaching workload includes both teaching with exercises and lectures exclusively in English in the master's program "Microbiology and Virology", specialty "Pharmacy" at Sofia University "St. K. Ohridski", as well as supervision of 1 bachelor's and 1 master's thesis.

Lora Simeonova's high general, professional and linguistic culture define her as a sought-after partner for participation in scientific projects, a beloved lecturer, an active reviewer and a participant in the scientific and public life of the Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences.

It is my pleasure to know Lora Simeonova personally as an excellent student, and later - as a thorough, precise researcher, with enduring interests in virology, which is also proven by her scientific output.

In conclusion, the comprehensive assessment of the candidate's research and teaching activities, her contributions and participation in developing and promoting scientific achievements in her field, her yearslong experience in teaching modern virology and antiviral therapy, as well as her personal qualities, give me the confidence to vote positively for the appointment of Chief Assistant Professor Dr. Lora Simeonova Simeonova, to the academic position of "Associate Professor" at the Institute of Microbiology "Stefan Angelov", BAS, Sofia. Her achievements exceed the minimum required for "Associate Professor", so I confidently suggest that the members of the respected scientific jury also vote positively.

Sofia,

REVIEWER:

June 5th, 2025

(Prof. DSc. Radka Argirova)