



TO THE CHAIRMAN  
OF THE SCIENTIFIC JURY DESIGNATED  
BY ORDER NO. 521/15.12.2022.  
OF THE DIRECTOR OF THE NCIPD

## STATEMENT OF OPINION

**Reviewer: Prof. Raina Tzvetanova Gergova, MD, PhD,**  
Head of Department of Medical Microbiology, Faculty of Medicine,  
Medical University - Sofia, e-mail: [rtgergova@gmail.com](mailto:rtgergova@gmail.com)  
Scientific specialty 01.06.12 Microbiology  
Outer member of the Scientific Jury

**Regarding: The announced competition for "ASSOCIATE PROFESSOR",**  
State Gazette, No. 87/01.11.2022, page 77, for the needs of the Department of Microbiology, NCIPD,  
in the field of higher education 4. "Natural sciences, mathematics and informatics"  
**and professional direction 4.3 Biological Sciences,**  
**in the scientific specialty 01.06.12 "Microbiology"**

**with a candidate: Chief Assistant IVA PETROVA TRIFONOVA, PhD**  
and Head of the National reference laboratory (NRL), NCIPD,  
"Vector-borne infections, leptospires and listeria"

I declare, that I have no conflict of interest with the participant in the competition.

The statement of opinion has been drawn up in accordance with the requirements of the Regulations for the implementation of the the Development of the Academic Staff of the Republic of Bulgaria Law.

The submitted documents and materials for the competition, by the only one candidate, Chief Assistant Iva Petrova Trifonfova, PhD, have been carefully classified and fully meet according to the requirements and the criteria of the Development of the Academic Staff of the Republic of Bulgaria Low and the Regulations for its implementation, as well as NCIPD criteria and the regulations for awarding and occupying the academic position "Associate professor". The participant in the competition certifying 12 years of the candidate's employment status in the NRL "Vector-borne infections, leptospires and listeria", NCIPD, of which 10 years as chief assistant by using the presented report. The candidate shows that it meets and even exceeds twice the minimum requirements (400 points), demonstrated 795 points with whom she participates in this competition.

### **Career development of the candidate**

Chief Assistant Iva Trifonova graduated in 2005 as a **bachelor's degree in Molecular Biology**, in the Faculty of Biology of Sofia University "St. Kliment Ohridski". In 2007, she defended her **master's degree in molecular virology**, in the Faculty of Biology of Sofia University "St. Kliment Ohridski". Thesis topic was: "Serological study of anti-cytomegalovirus immunoglobulins G and M with the ELISA method". In 2008-2010, she was a **full-time doctoral student at NCIPD**, Department of Microbiology, NRL "Vector-born infections, leptospires and listeria". She obtained the Educational and Scientific Degree of **Doctor of Microbiology in 2011** after defending her dissertation on the topic "Optimization of serological diagnostics and studies on Lyme borreliosis pathogenesis with recombinant antigens from *Borrelia burgdorferi*". She has consecutively worked as a biologist (2010-2012) and chief assistant (2012-2020), and has become the Head of NRL laboratory "Vector-born infections, listeria and leptospires" of NCIPD, Department of Microbiology, since 2020. The professional qualification of the candidate is based **on two medical specialties - 01.06.12 "Microbiology", acquired in 2017 and the second 01.06.13. "Virology"**, in which the specialization continues and will soon be finalized, as well as many thematic courses in Bulgaria and in other European countries, aimed at mastering various diagnostic methods in microbiology and virology, as well as in connection with the refinement of experimental laboratory work with the most modern methods for gene expression, sequencing and whole-gene sequencing.

**The teaching activity** during the last 5 years (2018 - 2022) averaged 23.73 hours per year. She actively takes part in the teaching activities of the Department of Microbiology in postgraduate training courses, consulting, methodical and experimental-organizational activities of the enrolled specialists, graduates with lectures, exercises and seminars, in accordance with the program of the NCIPD. Fluent in written and spoken English.

### **Research activity**

Chief Assistant Iva Trifonova participated in the competition with **48 scientific papers**, of which **22 were described with an Impact factor** and of them **21 with the following dissertation defense**, and the achieved **Impact factor was 35.211**. The candidate is the first author of 6 of the presented publications. There are a total of 26 publications in Bulgarian scientific publications, 13 of them before the defense of the dissertation and the remaining 12 are the following, with 1 being the participation as a co-author of the chapter of the book. Extremely high scientific activity shows the participants in the competition by presenting participation in the congress - 27 abroad and 80 in Bulgaria. Impressively, **one article is in Emerging Infectious Diseases with an Impact factor of 6.751, and eight of the articles are in Descriptions with an impact factor of over 2** (Vector-Borne and Zoonotic Diseases; Journal of Medical Virology, 2 articles; Ticks and Tick-borne Diseases ;

Emerging Infectious Diseases; Infection, Genetics and Evolution; Journal of Infection and Public Health; Journal of Clinical Virology).

The scientific-research activity and scientific-metric indicators of the candidate are at least two times more than the minimally required publications for a competition for "Associate professor" in Bulgaria. She presents the following scientific works:

- Dissertation work and abstract to it.
- Publications in periodical scientific journals with an impact factor (No.1-22)
- Participation in the author's collective of the head of book (No.23)
- Publications, before the defense of the dissertation work in Bulgarian scientific journals (No.24-33)
- Publications after the defense of the dissertation in Bulgarian scientific journals (No.34-48)
- Participation in scientific forums (No.1-107 congress papers)

73 citations (without self-citations of all authors) of the scientific works were noted, mostly in foreign journals, after the defense of a doctoral dissertation, and a total of 146 are found in the recognized databases with scientific information, much more than the 50 required for the competition.

The scientific researches of Iva Trifonova are mainly in the field of infectious diseases and are aimed at current and significant problems in microbiology and virology. These are mainly the causative agents and vectors of viral and bacterial transmissible infections, as well as other rarer but medically relevant bacteria and viruses. The results, analyzes and conclusions contain valuable information about clinical-therapeutic behavior and epidemiological measures both for our country and for other countries with similar problems. Numerous scientific research methods were used, both classic serological (PCR, ELISA, immunoblot) and the most modern molecular-genetic ones such as: conventional PCR, nested PCR, PCR with reverse transcription, Real Time PCR, phylogenetic analysis and whole genome sequencing.

Important new information is provided by studies on birds and animals as reservoirs of the causative agents of Lyme borreliosis and Human granulocytic anaplasmosis, Dobrava hantavirus (DOBV) and Puumala hantavirus by PCR. Sequencing and cluster analysis of isolates leptospire were done. The frequency of the vectors of the infections - the Crimean-Congo hemorrhagic fever virus in ticks and the West Nile virus in mosquitoes - was also studied. Other important studies are in patients with West Nile fever and Crimean-Congo hemorrhagic fever, of hantavirus infections in patients with hemorrhagic fever with renal syndrome, and of hospital-acquired bacterial and viral infections with unexplained febrile conditions.

### **Evaluation of contributions**

Most of the candidate's contributions are both of scientific-theoretical and scientific-applied value. The following can be highlighted as the most important:

1. **Survey of reservoirs of dangerous transmissible infections** show extremely interesting results for epidemiology. Studies cover: bacterial agents of transmissible infections such as *Borrelia burgdorferi* and *Anaplasma spp.*; other bacteria such as *Leptospira* and *Listeria spp.*; viral agents of

transmissible infections including West Nile virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus and many other viruses. The etiology of tick-borne infections in rodents was established, mainly infected with *Borrelia burgdorferi* (22.5%) and *Anaplasma phagocytophilum* (8.8%). The blackbird and great tit have been established as reservoirs of Lyme disease and West Nile fever. *Leptospira spp.* was found in nearly 1/3 of rodent samples (28.4%) in three regional countries (Pazardjik, Plovdiv and Smolyan). Hantaviruses have also been detected in many rodents, Dobrava hantavirus detected in >7% of one species of mouse and Puumala hantavirus detected in another species in three regions of the country. A very high (72%) seroprevalence of specific antibodies against the Crimean-Congo hemorrhagic fever virus was shown in Bulgarian domestic ruminants. The conclusions of the studies give correct directions to the epidemiological undertakings in the regions affected by these dangerous agents and their reservoirs.

**2. Pioneers for the country studies of the vectors of viral and bacterial transmissible infections with modern genetic methods.** The spread of Crimean-Congo hemorrhagic fever virus in ticks and West Nile virus in mosquitoes was studied, using PCR product sequencing and the most advanced and informative method whole-genome sequencing

**3. Development of new diagnostic methods that fill gaps in the diagnosis of important infections.** Molecular tests have been introduced for the first time in our country to prove many viruses such as those of West Nile fever, tick-borne encephalitis, Dengue viruses, Yellow fever, Zika and Toscana viruses, etc. In a comparative study, the advantages of the ELISA and the immunoblot method in the diagnostic possibilities of PCC were substantiated. Using whole genome sequencing of West Nile virus, its clonal characteristics have been determined. Diagnostic methods for leptospirosis and listeriosis have also been studied and introduced. Different variants of ELISA for leptospirosis were compared. Clinical isolates of *Listeria monocytogenes* were serotyped. A system for cloning and expression of *B. burgdorferi* immunodominant proteins has been developed. 4 ELISA tests with recombinant protein antigens of *B. burgdorferi* were developed and studied for the first time in our country and their superiority over ELISA tests with antigen from a whole bacterial cell of *B. burgdorferi* was proven. The new introduced design of the developed ELISA has high sensitivity in the different stages of the disease and it can be used for reliable serological diagnosis of Lyme borreliosis.

**4. Study of spread of infections with difficult to prove etiology.** A very important contribution is the etiological study of acute unclear febrile conditions in Bulgarian patients. The Crimean-Congo hemorrhagic fever virus was found as the causative agent in about 4% of the investigated cases, most often in Burgas and Sliven, and in about 3% of the hantaviruses, mainly in the regions of Burgas and Plovdiv. During a study of the distribution of Dobrava and Puumala hantaviruses, the causative agents of Hemorrhagic Fever with Renal Syndrome, the participation of Puumala hantavirus as a cause of diseases in our country was established for the first time. The areas with the highest seroprevalence of West Nile virus in Sofia and Vidin areas (>7%) and Crimean-Congo hemorrhagic fever virus in

Yambol and Haskovo were also established. The high serological frequency of Toscana virus, the causative agent of Sandfly fever, proven in 2019 in different geographical regions, in Blagoevgrad, Kardjali, Yambol, Varna and Pleven, is striking. The circulation of the virus is risky for causing vague fever and meningitis, especially in summer and in the southern regions of the country. The leading causative agents of leptospirosis have been identified in the most affected regions of Pazardzhik, Sofia, Montana, Shumen and Burgas in our country, with *Leptospira icterohaemorrhagiae* (in about 2/3 of cases) and *Leptospira romona* (in about 1/5) being the most frequently detected.

5. **First for Bulgaria study of the viral load in the COVID-19 infection.** A large-scale study included 123 healthcare workers after administration of RNA and vector vaccines against the virus that causes COVID-19 to investigate their post-vaccine humoral and cell-mediated immune response. Another study investigated the role of viral load in patients with COVID-19 and found an association of severe clinical manifestation with higher SARS-CoV-2 viral load, older age, higher levels of IgA-antibodies and cytokines IL-1 $\beta$ , IL-10 and IL-18. The information obtained in these studies is again very valuable for prophylactic, prognostic and therapeutic behavior in the treatment of this disease.

### **Conclusion**

The candidate Chief Assistant Iva Trifonova presents scientific-metric indicators that not only meet the requirements, but also exceed the criteria of the implementation of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for its implementation for as well as the criteria and rules of the NCIPD for the awarding and occupying the academic position of "Associate Professor".

Her studies cover undeveloped important topics of infectious pathology in our country and have important scientific theoretical and scientific applied importance for microbiologists, epidemiologists, infectious disease specialists and other clinical specialties. Established classic and the newest molecular-genetic methods were used in the implementation of developments.

The candidate has the educational and teaching activity and collaboration with foreign specialists, successfully participating in a significant number of internationally funded projects. The upward career development of the candidate is indicative of her diligence in work, professional qualities and ambition.

Based on everything stated above, I most strongly support the candidacy of the participant in the competition and propose to the other members of the scientific jury to award the academic position of **ASSOCIATE PROFESSOR** to **Iva Petrova Trifonova, PhD** for the needs of the "Microbiology" Department of the NCIPD.

31.01. 2023.

Reviewer:  
Prof. Raina Tzvetanova Gergova, MD, PhD