

To the Chairman of the Scientific Jury
on the announced competition for "Associate Professor"
in the scientific specialty 01.06.12 "Microbiology"
for the needs of the Department of Microbiology, NCIPD
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REVIEW

of Prof. Lyudmila Boyanova Georgieva, Ph.D., D.M.Sc.
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Scientific specialty - microbiology

Member of the Scientific Jury

appointed by Order No. 521/15.12.2022

by the Director of the NCIPD

Regarding
the Competition for the academic position "Associate Professor"
in the higher education field
4. "Natural sciences, mathematics and informatics"
and professional direction
4.3. "Biological Sciences", Scientific specialty 01.06.12 "Microbiology" for the
needs of the "Microbiology" Department
of the National Center of Infectious and Parasitic Diseases (NCIPD)
with a candidate:

Chief Assistant **IVA PETROVA TRIFONOVA**,
PhD of microbiology and Head of the NCIPD laboratory,
National reference laboratory (NRL) "Vector-born infections, listeria and
leptospires"

I declare no conflict of interest with Chief assistant Iva Petrova Trifonova, PhD.

All submitted documents of the candidate have been prepared according to the requirements of the Development of Academic Staff in the Republic of Bulgaria Act and the rules and criteria of the NCIPD for the academic position of "Associate professor". She meets the minimum requirements (400 points), exceeding them almost twice with a total number of 795 points for the current competition.

BRIEF BIOGRAPHICAL DATA AND PROFESSIONAL DEVELOPMENT

Chief assistant Iva Trifonova has a motivated and upward professional development. She graduated with a bachelor's degree in molecular biology in the Biological Faculty of Sofia University "St. Kliment Ohridski" in 2005, with a master's degree in molecular virology in the same faculty in 2007 and was full-time doctoral student at NCIPD, Department of Microbiology, NRL " Vector-born infections, listeria and leptospire" in 2008-2010. In 2011, she received the educational and scientific Ph.D. "Doctor of Microbiology" degree after defending her thesis "Optimization of serological diagnostics and studies on Lyme borreliosis pathogenesis with recombinant antigens from *Borrelia burgdorferi*". In 2017, she received a specialty in Microbiology, and since 2021, she has started specializing in clinical virology.

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 leptospire" of NCIPD, Department of Microbiology, since 2020. I am impressed by the participation of Iva Trifonova in a very large number of courses, trainings and specializations, which shows her striving for constant self-improvement. She specialized at Aristotle University of Thessaloniki, Greece in 2010 and in Istituto Superiore di Sanita in Rome, Italy in 2017, and participated in courses, seminars and webinars at home and abroad, among them in Sweden in 2016, in Italy in 2017, in Denmark in 2018 and in three Emerging Viral Diseases-Expert Laboratory Network (EVD-LabNet) webinars in 2022.

The main topics of her training and development are molecular methods for diagnosis of viral and bacterial infections, mainly zoonoses, incl. by systems for gene expression, sequencing and analysis and whole genome sequencing.

RESEARCH ACTIVITY

Most of Iva Trifonova's research activities are in a specific field of infectious diseases, which is of great clinical, therapeutic and epidemiological importance for our country and on a global scale. These are mainly viral and bacterial causative agents of infections transmissible to humans as well as other medically important bacteria and viruses. The studies focus on:

- the bacterial causative agents of transmissible infections: *Borrelia burgdorferi* sensu stricto, *Borrelia burgdorferi* sensu lato complex, *Anaplasma* spp.,
- viral causative agents of transmissible infections: West Nile virus (WNV), Crimean-Congo hemorrhagic fever virus, yellow fever virus,
- other bacteria such as *Leptospira* and *Listeria* spp.,
- other viruses such as Dobrava-Belgrade hantavirus, Puumala hantavirus and the causative agent of COVID-19 pandemic SARS-CoV-2, etc.

Numerous research methods have been used such as:

- complement fixation test, immunoblot and ELISA for IgM and IgG antibodies, serotyping,

- conventional PCR, nested PCR, Reverse Transcription PCR, Real Time PCR, PCR product sequencing and phylogenetic analysis, and whole-genome sequencing (of West Nile virus).

A system for cloning and expression of *B. burgdorferi* immunodominant proteins was developed and original PCR techniques were elaborated for the bacterial genes and plasmids.

ELISA tests with recombinant *B. burgdorferi* OspC, FlaB, OspA and VlsE protein antigens were performed and implemented for the first time in our country. They exhibited high specificity and sensitivity and better accuracy than that of ELISA tests with whole-cell borrelial antigen.

Extensive studies on birds or animals as reservoirs of the causative agents of Lyme borreliosis and Human granulocytic anaplasmosis were performed by PCR and ELISA for antibodies, Dobrava hantavirus and Puumala hantavirus were investigated by PCR, sequencing and clustering of isolates, and leptospire were evaluated by PCR. Prevalence of Crimean-Congo hemorrhagic fever virus in tick vectors and that of West Nile virus in mosquitoes was also a topic of investigation.

Other important studies are the complex evaluation of patients with West Nile fever and Crimean-Congo hemorrhagic fever, of hantavirus infections in patients with hemorrhagic fever with renal syndrome, and of bacterial and viral infections in patients with obscure febrile conditions.

RESEARCH PUBLICATIONS, CONGRESSES AND PROJECTS

Iva Trifonova participates in the competition with **48 scientific papers** as follows:

- 22 publications in scientific journals with impact factors, almost all of the articles (21) being published after the PhD dissertation defense;
- a co-authorship of a chapter in a book by Arbilis publishing house, 2022 and
- 25 publications in Bulgarian scientific journals, of which 15 after the PhD dissertation defense.

Of all scientific works, a total of 37 were published after the year of dissertation. In the publications, the wide range of methods (epidemiological, immunological and molecular) is impressive. In addition, a large number of microorganisms were evaluated, among them bacteria, the causative agents of Lyme borreliosis, human granulocytic anaplasmosis, hantaviruses and the numerous causative agents of viral transmissible infections, and recently, the causative agent of COVID-19, which shows her quick reaction to the most alarming infection during the recent years.

Notably, all 21 post-dissertation publications are in **journals with impact factors**, many of them international (see below). The candidate is the first author of 6 of the publications.

Iva Trifonova also has a constant presence in Bulgarian medical literature, with 15 articles in Bulgarian medical journals after the PhD dissertation defense and participation in a chapter of the book dedicated to the COVID-19 pandemic "Viral load, dynamics of

specific antibodies and levels of the main cytokines depending on the severity of COVID-19".

Participation of Iva Trifonova in international **scientific congresses and forums** (a total of 27, of them 19 after the PhD dissertation defense), as well as in a large number of scientific forums in our country (a total of 80, of them 63 after the dissertation defense) is another indisputable indicator for her research activity.

The candidate has participated in **research projects/programs**, of which 4 with international funding, 5 funded by the National Fund for Scientific research and a national program. This is a total of 10 projects/programs, which is important for the cooperation with our and international researchers from many European and other countries. Here I cannot fail to highlight the organizational and leadership role of Prof. Iva Hristova, with whom Iva Trifonova is in constant collaboration.

IMPACT FACTOR AND CITATIONS OF PAPERS

The total impact factor of Iva Trifonova's publications is over 35, which confirms their contribution to and importance in the international medical literature.

Again, I want to emphasize that all of her 21 publications published after the PhD dissertation defense are articles in **journals with an impact factor**. Impressively, eight of the articles are in journals with an impact factor above 2 and one is in Emerging Infectious Diseases with an impact factor of 6.75 as follows:

- in Vector-Borne and Zoonotic Diseases in 2013,
- Journal of Medical Virology, two articles in 2015 and 2022,
- Ticks and Tick-borne Diseases in 2016,
- Emerging Infectious Diseases in 2016,
- Infection, Genetics and Evolution in 2019,
- Journal of Infection and Public Health in 2020 and
- Journal of Clinical Virology, in 2020.

The number of **citations** exceeds about three times (146 points) the minimum (50) required points for an associate professor. In the documents presented to me, a total of **73 citations** (excluding self-citations of the co-authors) of the candidate's works are noted, most of them in foreign journals, incl. journals with a very high impact factor such as Frontiers in immunology, Eurosurveillance, Frontiers in Microbiology, Emerging Microbes & Infections, etc. The citations once again show the reflection of Iva Trifonova's research work in the medical community and the interest in and benefits of her studies on a national and international scale.

CONTRIBUTIONS

Among Iva Trifonova's contributions, I will focus on those that impressed me the most. Most contributions are of both scientifically theoretical and applied value, so I do not separate them in this way. Of all contributions, I will highlight those that fill understudied or still non-evaluated topics of infectious pathology in the country:

1. Studies on infection reservoirs. Frequency of the causative agents of tick-borne infections in rodents was determined, most often (22.5%) of *Borrelia burgdorferi* and 8.8% of *Anaplasma phagocytophilum*, with the most common reservoirs being the yellow-throated wood mouse (*Apodemus flavicollis*) and the field mouse (*Apodemus agrarius*), respectively. Hantaviruses have also been found in rodents, with Dobrava hantavirus detected in >7% of *A. flavicollis* mice and Puumala hantavirus found in bank voles (*Myodes glareolus*) in three regions of the country. *Leptospira* spp. were detected in over ¼ (28.4%) of rodent samples in three regions (Pazardjik, Plovdiv and Smolyan). Priority is also given to the serological examination of birds as causative agents of Lyme borreliosis and West Nile fever. The blackbird and the great tit have been found to be main reservoirs of the infections. It is epidemiologically important that specific antibody seroprevalence against Crimean-Congo hemorrhagic fever virus in domestic ruminants is very high (72%). This points to the need to better control tick populations.

PCR and ELISA methods were used in the studies, nested PCR was applied for *Leptospira* spp. and phylogenetic analysis of Puumala hantavirus sequences, and a newly developed TaqMan Real time RT was used for hantaviruses.

Most studies are a priority for the country. The results and newly introduced methods are useful for faster detection of infections or epidemics, especially the regions defined in the studies.

2. First studies for Bulgaria on the vectors of viral and bacterial transmissible infections. The prevalence of the Crimean-Congo hemorrhagic fever virus in ticks and that of West Nile virus in mosquitoes was studied by PCR, sequencing and the most advanced and informative method, the whole-genome sequencing.

3. Diagnostic methods. Molecular tests have been introduced for the first time in our country to detect numerous viruses, such as those of West Nile fever, tick-borne encephalitis, dengue, yellow fever, Zika and Toscana viruses, etc. Diagnostic parameters of serological tests for antibodies to hantaviruses were studied and higher accuracy of the ELISA and immunoblot method was found compared with that of complement-binding reaction. By sequencing the genome of West Nile virus, its clonal characteristics have been determined.

Diagnostic methods for leptospirosis and listeriosis have been investigated as well. When comparing the reference microscopic agglutination test with ELISA for diagnosis of leptospirosis, good accuracy of ELISA was found for samples taken at the beginning of the infection. *Listeria monocytogenes* isolates from clinical samples were serotyped.

Four ELISA tests with peptide iR6 (C6) antigens from the Vlse region proteins of 3 borrelial causative agents of Lyme disease were developed. The humoral response to synthetic C6 peptide antigens was investigated and the C6 ELISA was found to be highly sensitive at different stages of the disease, and also a cheap and easy-to-perform test. Therefore, the test can be routinely used for serologic diagnosis of Lyme disease.

All of these diagnostic tests and results undoubtedly fill an important and understudied diagnostic niche in infectious pathology and are beneficial for the diagnosis of infections.

4. Prevalence of infections. A very significant contribution is the etiological study of obscure febrile conditions in the country. The Crimean-Congo hemorrhagic fever virus was found to be the cause of the conditions in about 4% of examined groups, most often in Burgas and Sliven, and hantaviruses were detected in about 3% of them, mainly in Burgas and Plovdiv regions. During a study on Dobrava and Puumala hantavirus prevalence, the causative agents of hemorrhagic fever with renal syndrome in Bulgaria, Puumala hantavirus was established as a causative agent of the disease for the first time in the country.

Areas with the highest (>7%) seroprevalence of West Nile virus were determined in Sofia and Vidin regions and those of Crimean-Congo hemorrhagic fever virus were Yambol and Haskovo regions.

The prevalence of Toscana virus, the causative agent of Sandfly fever, was high in Bulgaria, the virus was serologically proven very often (in about 24%, ranging from 4.4 to 53.5%) in healthy adults from different geographic regions in 2019. Circulation of the virus as a possible cause of febrile illness and meningitis should be considered, especially in summer and in southern country regions. In Bulgaria, the highest seroprevalence was found in the regions of Blagoevgrad, Kardzhali, Yambol, Varna and Pleven.

The leading causative agents of leptospirosis (most often *Leptospira icterohaemorrhagiae* in about 2/3 of cases and *Leptospira pomona* in about 1/5 of cases) have also been identified. The most affected regions have been Pazardjik, Sofia, Montana, Shumen and Burgas.

5. Viral load study in COVID-19 infection. Humoral and cell-mediated immune response of 123 healthcare workers after vaccination for COVID-19 with RNA and vector vaccines was investigated. Another study focused on the viral load in patients with COVID-19 and revealed an association between severe clinical diseases and older age, higher viral load, higher levels of the cytokines (IL-1 β , IL-10 and IL-18) and IgA-antibody levels.

EDUCATIONAL AND TEACHING ACTIVITY

Iva Trifonova takes part in the educational and teaching activities of the NCIPD Department of Microbiology, incl. in courses for postgraduate training, as well as with consulting, methodical and experimental/organizational activity, according to the NCIPD program. In 2018-2022, she had an average teaching workload of 23.7 hours.

CONCLUSIONS

Scientific production of Iva Trifonova covers and, in many respects, (publications, impact factors, citations and project participation) exceeds the criteria of the Development of Academic Staff in the Republic of Bulgaria Act and the Regulations for its implementation,

as well as NCIPD criteria and the regulations for awarding and occupying the academic position "Associate professor".

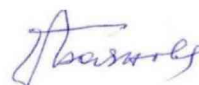
Her works have both scientifically theoretical and applied importance not only in microbiology, but also for specialists in epidemiology and infectious diseases. In addition, they cover understudied but important topics of infectious pathology in the country. Classical and modern techniques such as whole-genome sequencing have been used to achieve the research aims.

She has educational and teaching activities and collaborates with foreign specialists, participating in internationally funded projects. I would recommend her to organize more thematic postgraduate courses and to prepare a monograph on the topics she is working on.

All presented documents and analysis of the results convince me that Iva Trifonova is a modern and very active specialist and researcher and, as I started with, she has a motivated and upward professional development.

That is why I most strongly support her candidacy and suggest that the members of the Scientific Jury award the academic position of ASSOCIATE PROFESSOR for the needs of the NCIPD Microbiology Department to the Chief Assistant **Iva Petrova Trifonova**, Ph.D.

Sofia, January 29, 2023



Prof. Lyudmila Boyanova Georgieva, Ph.D., D.M.Sc.