

To the attention of the scientific jury
determined by order No. 521 of 15.12.2022
of the Director of the National Center for
Infectious and Parasitic Diseases, Sofia

STATEMENT

FROM: Prof. Dr. Magdalena Ivanova Leseva, PhD

Head of Microbiology Laboratory at UMBALSM "N. I. Pirogov"

ON: Participation of chief assistant Iva Petrova Trifonova in the Competition for the occupation of the academic position "Associate Professor" in the scientific specialty "Microbiology", for the needs of the "Microbiology" department of NCIPD, announced in SG no. 87 of 01.11.2022

Autobiographical data

Since 2010 and currently chief. assistant Iva Trifonova works at the National Center of Infectious and Parasitic Diseases, Department of Microbiology, NRL Vector-borne Infections, Leptospire and Listeria: In the period 2010-2012 as a biologist, then as a chief assistant. Since 2020, she is the head of the laboratory. In 2011, she obtained the educational and scientific degree of Doctor of Microbiology, defending a dissertation on the topic "Optimization of serological diagnostics and studies on the pathogenesis of Lyme borreliosis with recombinant antigens from *Borrelia burgdorferi*". In 2017, she acquired a specialty in microbiology, and from 2021 she specialized in clinical virology. The candidate has many years of experience in research work with vector-borne bacterial and viral pathogens of infectious diseases. She knows and applies the basic molecular-genetic techniques, as well as serological methods for diagnosis. The candidate participates in a large number of research projects.

In the competition, Iva Trifonova presented a total of 156 scientific works, of which:

- **22 scientific publications in foreign editions and editions with an impact factor**, of which 1 before and 21 after the defense of the doctoral dissertation.
- **26 scientific publications in Bulgarian journals**, of which 10 before and 16 after the defense of the doctoral dissertation.

**Significance of scientific and scientific-applied contributions
of chief assistant Iva Trifonova**

I. DISSERTATION CONTRIBUTIONS. I fully agree with all the contributions of the dissertation mentioned by the author.

A. For the first time in our country, systems were developed for efficient cloning and expression of the main immunodominant proteins of *B. burgdorferi* – OspC, FlaB, OspA and VlsE. The participation of each of the recombinant proteins in the development of an antibody response in the different phases of Lyme borreliosis was studied. The knowledge about the possible serological findings during the course of the disease has been expanded.

B. Original polymerase chain reaction systems have been developed to amplify the entire genes encoding the OspC, FlaB, OspA and VlsE proteins, which can be applied to demonstrate the genome of *B. burgdorferi*; The plasmid vectors that provide efficient cloning of the *ospC*, *flaB*, *ospA* and *vlsE* genes of *B. burgdorferi* are specified; The appropriate types of chemocompetent cells allowing successful transformation and ensuring efficient expression of the recombinant proteins were experimentally established.

C. The first ELISA tests in Bulgaria were developed with the recombinant OspC, FlaB, OspA and VlsE protein antigens, with very high specificity and sensitivity, comparable to that of commercial whales and higher than that of ELISA tests with whole-cell *Borrelia* antigen; The feasibility of ELISA tests with each or with different combinations of the recombinant proteins OspC, FlaB, OspA and VlsE as the antigen for detection of specific antibodies in various phases of Lyme borreliosis has been explored.

II. SCIENTIFIC CONTRIBUTIONS FROM THE OTHER STUDIES:

1. For the first time, infection with **Dobrava hantavirus** (DOBV) was detected by PCR method in different species of mice - *Apodemus flavicollis* (7.7%) and *Apodemus agrarius* (1.43%) in three regions of the country;
2. The circulation of **Puumala hantavirus** (PUUV) in the country has been proven for the first time using molecular genetic methods.
3. The country's first study of rodents for infection with pathogenic **leptospire**s was conducted.
4. Pioneering for the country PCR study of ticks of the species *Hyalomma marginatum* and *Rhipicephalus sanguineus* for the presence of infection with the **Crimean-Congo hemorrhagic fever** (CCHF) virus.

Arrangement of publications by place in the author's collective:

- Single or first author – 6 (12.5%);
- Second author – 9 (18.75%);
- Third and after third author – 33 (68.75%).

• ***1 chapter in a monograph***

• Scientific works presented at scientific congresses, conferences, symposia, of which:

- ***27 abroad***
- ***80 in Bulgaria***

Reference for citations and impact factor:

- The candidate indicated 73 publication citations, no self-citations.
- Total impact factor of publications 35.211.

From all this, I conclude that the presented scientific production is sufficient in terms of volume and quality for participation in the competition.

**Main directions of scientific research activity
of chief assistant Iva Trifonova**

- Ecological-epidemiological studies;
- Pioneering studies for the country on vectors of viral and bacterial transmissible infections;
- Studies on viral hemorrhagic fevers, including pioneering for the country seroepidemiological studies on the spread of viral hemorrhagic fevers and vector-borne infections in the country;
- Studies on leptospirosis in Bulgaria;
- First studies in the country on the antibody response against synthetic peptides in Lyme borreliosis
- Country-leading studies on viral load in patients with COVID-19 infection
- Investigations of patients with unexplained febrile conditions and unspecified meningitis.

The scientific works reflect results of in-depth studies, most of which are pioneering for the country and related to the introduction of a number of new methods for research of the causative agents, vectors and reservoirs of the main vector-borne bacterial and viral zoonoses in our country. The candidate's broad range of scientific interests makes an impression.

5. The presence of the **West Nile fever** virus in *Culex pipiens* mosquitoes from Pleven and Ruse regions has been proven. For the first time in the country, conventional and Real-time RT-PCR systems have been introduced for the detection of West Nile virus (lineages 1 and 2) (WNT), Tick-borne encephalitis (TE), Zika virus, Dengue fever, Yellow fever, Toscana virus, Usutu virus and Chikungunya. West Nile virus genome was sequenced for the first time and lineage 2 of central/southern lineage was determined.

6. In-depth studies on viral hemorrhagic fevers have been carried out, and the sero-epidemiological studies on their prevalence are pioneering for the country.

7. The author's studies on leptospirosis in Bulgaria prove that the ELISA test is well applicable for testing samples taken at the beginning of the disease, while during the convalescent period the sensitivity of the test decreases. It was established that the leading serogroups causing leptospirosis in the country are *Leptospira icterohaemorrhagiae* (66.07%) and *Leptospira pomona* (19.64%).

8. The country's pioneering studies on the viral load in patients with COVID19 infections are relevant. It was established that the viral load is inversely dependent on the serum antibody response, as well as the presence of a relationship between the severity of the disease and the dynamics of the viral load and the antibody response.

Other areas of Iva Trifonova's research include:

9. Serotyping of listeriosis;

10. First studies in the country on the antibody response against synthetic peptides in Lyme borreliosis;

11. Very valuable studies on patients with unclear febrile conditions and unspecified meningitis to clarify the etiological structure of the causative agents of acute CNS infections.

Participation in research projects of chief assistant Iva Trifonova

Chief Assistant Iva Trifonova participated in the research teams of a total of 10 research projects, four of which were internationally funded, five were funded by the National Fund for Scientific Research and one national program.

Teaching activity of chief assistant Iva Trifonova

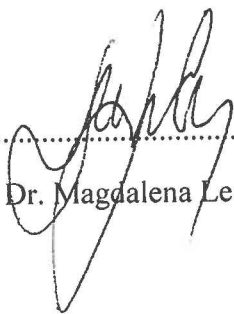
The average teaching load of Iva Trifonova for the period 2018-2022 is 23.73 hours.

Conclusion

Based on the analysis, I believe that Chief Assistant Iva Petrova Trifonova is a thorough scientist, with analytical thinking, whose achievements in the various directions in which she worked are of great importance for our country. I expect her to have a key role in the future innovative directions of development of the NCIPD. Therefore, I strongly recommend to the members of the esteemed scientific jury to vote positively and to propose to the Scientific Council of the NCIPD that chief assistant Iva Petrova Trifonova be elected to the academic position of "ASSISTANT PROFESSOR" in the scientific specialty "Microbiology" for the needs of the "Microbiology" department of NCIPD.

19.01.2023

Sofia


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(Prof. Dr. Magdalena Leseva, PhD)