

To the Chairman of the Scientific Jury,
appointed by Order № 196/30.06.2025 of
the Director of the NCIPD, Sofia

НАЦИОНАЛЕН ЦЕНТЪР ПО ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ	
Изм. №	20... г.
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Statement

By Assoc. Prof. Stefka Krumova Ivanova, PhD

Head of NRL Measles, Mumps, Rubella, Department of Virology at the National Center
of Infectious and Parasitic Diseases (NCIPD), Sofia

On a dissertation presented to a scientific jury formed by order № 196/30.06.2025 of the
Director of the NCIPD, for awarding the educational and scientific degree "DOCTOR" in
Professional field: 4.3. "Biological Sciences", Doctoral program in "Virology"

Dissertation topic: *Molecular-virological analysis of the transmission clusters of
introduced and prevalent HIV-1 subtypes in Bulgaria*

Dissertation author: Lyubomira Svilenova Grigorova

Scientific supervisor: Assoc. Prof. Ivailo Alexiev Ivanov, PhD

I declare that I have no common publications or any other conflict of interest with the
candidate, as defined in Article 4, paragraph 5 of The Law on Academic Staff Development in
the Republic of Bulgaria.

The documents of Lyubomira Svilenova Grigorova submitted to me for consideration
fully meet the requirements of the LPASB and the Regulations for its implementation, for the
award of the educational and scientific degree "Doctor"; therefore, I can formulate the following
opinion with full responsibility.

1. Relevance and significance of the dissertation topic

The topic considered in this dissertation is extremely relevant, given the global public
health problem represented by the HIV epidemic. HIV-1 is known for its extremely large
genetic diversity and diverse route of viral transmission. Despite all measures and efforts to
reduce the spread of the virus, including the development and constant updating of antiretroviral
therapy, HIV-1 continues its global spread. For this reason, it is necessary to thoroughly study
the transmission clusters formed in different genetic forms of the virus. The numerous
connections formed between risk groups show scientists worldwide that a detailed phylogenetic
study is necessary. This dissertation focuses on molecular biological methods, reconstruction

The dissertation includes the study of plasma samples from patients diagnosed in the National Center for HIV in the period from 1986 to 2020, divided into 3 groups. The first includes 663 sequences of patients diagnosed in the National Center for HIV, National Center for HIV Research, Sofia, Bulgaria for the period from 1986 to 2018 with established subtype B. The second group includes 270 sequences isolated from plasma samples of patients diagnosed in the National Center for HIV between 1995 and 2019, infected with HIV-1 CRF01_AE. And the third includes 1053 sequences of naive patients diagnosed in the period from 2012 to 2020. The studies were approved by the Ethics Committee of the National Center of Infectious and Parasitic Diseases and were partially funded by the project BG05M2OP001-1.002-0001 “Fundamental, translational and clinical research in the field of infections and infectious immunology”, funded by the OP “Science and Education for Smart Growth 2014 – 2020” and the European Regional Development Fund. The patients’ anamnestic data were provided by processing the self-assessment questionnaires that they filled out during the diagnostic process. Laboratory tests included a set of serological and molecular biological tests performed during the diagnostic process and virological monitoring of HIV patients in Bulgaria.

2.2. Results and Discussion

The results are written in three sections based on the study objects, with each section ending with an analysis of the transmission clusters by patient groups, which is extremely useful for subsequent conclusions. The “Results” chapter provides information on the possible routes of HIV-1 introduction into the country, the main affected patient groups, an analysis of the most common HIV-1 subtypes in Bulgaria (subtype B and the circulating recombinant form CRF01_AE), as well as a study of naive patients diagnosed with HIV-1 in Bulgaria in the period 2012 - 2020. The most modern methods were used for phylogenetic analysis and subsequent reconstruction of multiple phylogenetic trees, including one for subtype B, which provides a comprehensive molecular-epidemiological map of the circulating viruses in the country. The study outlines the main demographic and epidemiological profile of HIV-1-infected patients in Bulgaria, providing valuable information on the dynamics of the epidemic.

2.3. Conclusions and Contributions

At the end of the dissertation, the graduate student formulates 6 conclusions and 8 contributions, which I fully support. I recommend that the contributions be divided into those of a scientific-theoretical and those of a scientific-applied nature.

3. Publications related to the dissertation

The results of the dissertation have been published in 3 articles in refereed journals, with a total impact factor of 10.866. Two of the publications are in quartile Q1 and one in Q4, which fully meets the criteria for publication activity in the preparation of a dissertation work. In addition, the results of the studies have been presented at 18 national and two scientific forums abroad, which confirms the relevance of the topic.

The presented abstract complies with the requirements, fully reflecting the content of the dissertation work. It is written on 82 pages. It is illustrated with 24 figures and 5 tables.

4. Conclusion

In conclusion, I would like to summarize that the work provided to me is dissertable. The discussed topic is of an innovative and scientifically applied nature, and has the potential for subsequent enrichment and expansion of the focus of the experiments. The research was funded by a scientific research project, which confirms its scientific value.

Given the above, I believe that the dissertation fully meets the requirements of The Law on Academic Staff Development in the Republic of Bulgaria, the Regulations for its implementation, and the Regulations of the NCIPD for the Award of the Educational and Scientific Degree "Doctor". All required scientometric indicators have been met, which indisputably confirms the candidate's academic potential.

I give my positive assessment of Lyubomira Svilenova Grigorova's award of the educational and scientific degree "Doctor" in the scientific specialty „Virology."

Signature:

Assoc. Prof. Stefka Krumova Ivanova, PhD