

TO THE CHAIRMAN OF THE SCIENTIFIC JURY,
APPOINTED BY ORDER No. 276/06.10.2025
TO THE DIRECTOR OF THE NATIONAL CENTER
FOR INFECTIOUS AND PARASITIC DISEASES
PROF. DR. IVA HRISTOVA, PhD, DSc

STATEMENT

By

Prof. Dr. Nina Stoyanova Yancheva-Petrova, PhD

External member of the Scientific Jury

Head of the Department of Infectious Diseases, Parasitology and Tropical Medicine,
MF, MU-Sofia

Subject: dissertation for the educational and scientific degree "DOCTOR OF SCIENCE"

Field of higher education: 4. Natural Sciences. Mathematics and Informatics, Direction: 4.3. Biological Sciences. Scientific specialty: Virology

Author of the dissertation: Assoc. Prof. Ivaylo Ivanov Aleksiev, PhD

Department of Virology - National Center for Infectious and Parasitic Diseases

Topic of the dissertation: "Antiretroviral resistance and molecular epidemiology of HIV-1 in Bulgaria: An integrated analysis of genetic diversity, phylodynamics and demographic correlations"

In implementation of Order No. R-276/06.10.2025 of the Director of the National Center for Infectious and Parasitic Diseases, at the first meeting of the Scientific Jury held on 20.10.2025, it was agreed that I should present an opinion on the dissertation work for the educational and scientific degree "DOCTOR OF SCIENCE", on the topic "Antiretroviral resistance and molecular epidemiology of HIV-1 in Bulgaria: An integrated analysis of genetic diversity, phylodynamics and demographic correlations".

The statement is in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for its implementation, as well as with the Regulations for the Development of the Academic Staff of the National Center for Infectious and Parasitic Diseases.

Brief biographical data of the candidate for acquisition of the ESD "DOCTOR OF SCIENCES:

Assoc. Prof. Ivaylo Ivanov Aleksiev, PhD graduated from Sofia University "St. Kliment Ohridski" in 1994. In 2003 he started working at the National Reference Laboratory for HIV at the National Center for Infectious and Parasitic Diseases. In 2008, Assoc. Prof. Aleksiev acquired a specialty in "Clinical Virology". From 2008 to 2011 he was a PhD student at the National Center for Infectious and Parasitic Diseases, in 2011 he defended his dissertation for acquisition of the ESD "PhD", and in 2014 he was elected "Associate Professor". Since 2016, Assoc. Prof. Ivaylo Aleksiev has been the Head of the National Reference Laboratory for HIV, and since 2024 he has been the Head of the Virology Department at the National Center for Infectious and Parasitic Diseases.

Assoc. Prof. Ivaylo Aleksiev has conducted several specializations abroad and is a member of numerous national and international scientific organizations: Union of Scientists in Bulgaria, Bulgarian Society of Virology, European Society for Translational Antiviral Research (ESAR), Euroguidelines in Central and Eastern Europe Network Group (ECEE), European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

Relevance and significance of the topic of the dissertation:

After more than 40 years of the HIV pandemic, statistical data show that over 91 million people have been infected with the virus of HIV worldwide, leading to approximately 50 million deaths. By the end of 2024, 40.8 million people are living with HIV globally. About 1.3 million were newly diagnosed, and 630,000 died from HIV-related diseases in the same year. In Bulgaria, since 1986, when the first HIV infected person was diagnosed, over 4,700 people have been proved to be living with HIV. The combined antiretroviral therapy is effective in achieving an undetectable viral load in the blood of infected people, but cannot completely eradicate the virus from so-called "viral depots". With suboptimal adherence to antiretroviral therapy, viral strains that develop mutations with resistance to the antiretroviral regimen will have a fitness advantage and become dominant. The emergence of dominant resistant strains of HIV can lead to virological failure, and this type of drug resistance is called acquired resistance.

It has also been found that there is so-called transmission resistance, which is acquired during the initial infection, because it is already present in the HIV strain transmitted by the source of infection. In this sense, the topic of antiretroviral resistance and molecular epidemiology of HIV-1 in Bulgaria is of exceptional importance and relevance, as it has not only scientific value, but also practical value in the implementation and monitoring of treatment of people living with HIV in Bulgaria. To date, no such comprehensive analysis has been carried out for our country.

Structure of the dissertation:

The dissertation is developed on 310 pages, including the relevant appendices and bibliography. The content is structured according to the accepted requirements for scientific research and contains introduction, literature review, goal and objectives, materials and methods, results, discussion, conclusions, contributions, bibliography and applications. The dissertation work is illustrated with 43 tables and 58 figures.

The literature review includes 87 pages, in which Assoc. Prof. Aleksiev presents a brief description of the virus, with relevant historical data on retroviruses, biology and classification, structure of HIV, replication and life cycle. The antiretroviral drugs are presented, and the mechanisms of action of the individual groups are described in detail. Assoc. Prof. Aleksiev describes thoroughly the mechanisms of occurrence and the models of antiretroviral resistance, the available tests for its detection, and the interpretation of the results obtained. Transmission and acquired antiretroviral resistance, monitoring options, and recommendations for therapeutic behavior in the presence of resistance are discussed in detail. Other topics that Assoc. Prof. Alexiev discusses in the literature review are: origin, classification and nomenclature of HIV. Data are presented on the basis of global and regional distribution of the HIV-1 genetic variants, respectively the clinical and epidemiological significance of this genetic diversity. Assoc. Prof. Alexiev makes a reference from the genetic diversity of the virus to modern strategies for vaccine development. The evolutionary dynamics and future prospects are presented, which itself is a justification of the need for a detailed analysis of the circulating HIV-1 variants in Bulgaria as part of the global efforts to control the HIV epidemic. Assoc. Prof. Aleksiev presents in detail what exactly is phylogenetic analysis and as well as its two main types. The literature review ends with a conclusion about the justification of the relevance and the significance of the topic of the dissertation. Assoc. Prof. Aleksiev convincingly presents the need for detailed research in the field of the molecular epidemiology of HIV-1 in Bulgaria, the antiretroviral resistance and the practical application of these data in the clinical practice and in the development of prevention strategies in certain risk groups for infection.

The aim of the work is clearly defined, corresponding to the title of the doctoral thesis. 5 main tasks have been set to achieve the defined goal, with each task divided into additional subtasks - 21 in total.

The materials and methods used are presented in 63 pages. The main characteristics of the studied population and the sequenced samples are presented. The individual stages of the sequencing analysis, as well as the bioinformatic analysis and interpretation of the results, are clearly presented as well. The analysis of resistance by genotyping is presented in detail and clearly, as well as the factors that influence the assessment of resistance. A classification of the obtained mutations and their grouping by drug classes is presented. To be able to implement the tasks set in the dissertation work, Assoc. Prof. Aleksiev describes the clinical application of the data on the resistance mutations and the possible limitations. The subtyping and the identification of phylogenetic clusters are also comprehensively described. The choice of statistical methods for processing the obtained data is presented in a justified manner.

A separate chapter in the dissertation is an analysis of selected publications on the topic. Six publications have been analyzed, presenting molecular biological analyses of different transmission groups, subtypes and transmission resistance in antiretroviral therapy-naïve patients over an 8-year period.

The results are presented in 92 pages.

In the implementation of **task 1**, mutations associated with three classes of antiretroviral drugs have been identified and classified, and their impact on the effectiveness of the therapy have been analyzed. Cross-resistance within individual classes of antiretroviral drugs has been studied and the therapeutic response to these mutations has been predicted.

In the implementation of **task 2**, fragments of the pol gene of HIV-1 have been sequenced and analyzed from blood samples of 1654 individuals with HIV-1 infection diagnosed in Bulgaria between 1989-2023. Circulating HIV-1 subtypes and unique recombinant forms in the Bulgarian population have been identified, and their distribution has been analyzed. Molecular characterization of the evolutionary relationships between different HIV-1 isolates and analysis of the distribution and evolutionary dynamics of the main circulating subtypes have been performed.

In the implementation of **task 3**, reconstruction of phylogenetic trees, phylodynamic analysis and dating of the introduction of the main HIV-1 subtypes in Bulgaria using a molecular clock have been carried out. The spatio-temporal evolution and geographical distribution have been analyzed as well as the international origin of HIV-1 subtypes in Bulgaria through a global phylogenetic approach. Phylogenetic transmission clusters have been identified and

characterized, transmission networks and their distribution among different vulnerable groups have been studied.

In the implementation of **task 4**, the relationship between mutations associated with drug resistance and the demographic characteristics of infected individuals was studied. The distribution of HIV-1 subtypes and drug resistance by transmission categories and vulnerable groups, as well as in the different regions of Bulgaria, was analyzed.

Task 5 assessed the clinical relevance of identified resistance-associated mutations for the choice of antiretroviral therapy and national practices for monitoring drug resistance in HIV-1. Guidelines have been formulated to limit the spread of resistant strains among vulnerable populations.

The discussion is presented in 29 pages. Assoc. Prof. Alexiev discusses his results, comparing them with data from international studies on the demographic characteristics of the population studied and epidemiological trends, migration factors, special populations, antiretroviral resistance and their clinical significance, transmission and subtype-specific resistance.

The conclusions from the analyses performed are 21, divided into 5 groups.

The bibliographic reference includes 342 literary sources, of which 6 in Cyrillic, the remaining 336 in Latin. 16% of the sources are from the last 5 years.

Contributions of the dissertation:

Assoc. Prof. Ivaylo Aleksiev has presented 11 contributions of original scientific nature and 8 contributions of a scientifically applied confirmatory nature.

Abstract:

The abstract contains 125 pages and has been prepared in accordance with the corresponding requirements. It contains the necessary sections – introduction, aim, tasks, materials and methods, results, discussion, conclusions and contributions, publications related to the dissertation work.

Publications related to the dissertation work:

Assoc. Prof. Ivaylo Aleksiev has published 8 scientific articles in peer-reviewed international impact factor journals and one monograph. Total impact factor of the articles is 29.971, with 7 of the articles having quartiles in Q1, 1 in Q2.

Critical remarks and recommendations:

I have no critical remarks about the work of Assoc. Prof. Aleksiev. My recommendation is that the conclusions should be more synthesized and concise.

Conclusion:

The dissertation of Assoc. Prof. Ivaylo Aleksiev is up-to-date and original. It has been developed with detailed knowledge of the problem. The most extensive analysis of antiretroviral resistance and molecular epidemiology of HIV-1 in Bulgaria has been made, covering a 35-year period.

The results obtained have significant scientific value both for the country and globally. The analyses also have practical application regarding the monitoring of the antiretroviral treatment and the development of prevention strategies for risk groups.

I could state that Assoc. Prof. Aleksiev as an excellent clinical virologist, a prominent and well-known researcher in the international scientific community and an ethical colleague.

The dissertation fully complies with the Law on the Development of Academic Staff in the Republic of Bulgaria, the regulations for its implementation and the regulations of the National Center for Infectious and Parasitic Diseases.

I confidently give my **positive** assessment and strongly recommend that the esteemed scientific jury should award Assoc. Prof. Ivaylo Ivanov Aleksiev the educational and scientific degree of DOCTOR OF SCIENCES.

30.10.2025

Prepared by: /./

Prof. ~~Nina~~ Yancheva-Petrova, PhD