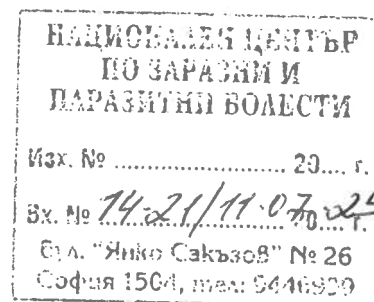


To the Chairmen of the Scientific Jury



## REVIEW / EVALUATION

**From professor Alexey Slavkov Savov, PhD., Head of the National Genetic Laboratory, SBALAG "Maichin dom" EAD. MU Sofia**

**Regarding:** PhD thesis of Lyubomira Svilenova Grigorova, a full-time PhD student at the Department of Virology of the National Center for the Study of Biological Sciences, Sofia, Bulgaria in the professional field 4.3. Biological Sciences, from the field of higher education 4. Natural Sciences, Mathematics and Informatics. The topic of the dissertation work is "Molecular-virological analysis of the transmission clusters of introduced and widespread HIV-1 subtypes in Bulgaria"

By order No. 196 of 30.06.2025 of the Director of the National Center for Infectious and Parasitic Diseases, Sofia, I have been selected as a reviewer in connection with the dissertation work of Lyubomira Svilenova Grigorova. During the defence procedure, all necessary materials have been submitted in accordance with the requirements of the "Regulations for the implementation of the law on the development of the academic staff in the Republic of Bulgaria of the National Center for Infectious and Parasitic Diseases"

**I declare that I have no conflict of interest with the author of the PhD thesis.**

### **Brief biographical data about the candidate.**

Lyubomira Grigorova graduated from the Master's degree in "Food Quality and Safety", Department of Microbiology, Faculty of Biology at Sofia University "St. Kliment Ohridski". Since 2020, she has been a full-time doctoral student at the Department of Virology of the National Center for the Study of Food Safety, Sofia, Bulgaria, with the scientific supervisor Assoc. Prof. Ivaylo Aleksiev Ivanov, PhD. In connection with the dissertation work, three publications have been published, two of which have an impact factor, which corresponds to the requirements of the "Regulations for the Development of Academic Staff". She has twenty participations in national and international scientific forums on the topic of the dissertation.

Molecular virological analyses play a fundamental role in understanding the dynamics of HIV-1 transmission in Bulgaria, where the epidemic is characterized by significant genetic diversity and evolving subtype patterns. By investigating transmission clusters and resistance mutations among both introduced and prevalent HIV-1 subtypes – such as B, A1 and A6 – infection pathways can be traced, high-risk networks identified and the emergence of drug-resistant strains detected. This approach not only improves epidemiological surveillance, but also informs targeted prevention strategies and optimizes antiretroviral treatment protocols tailored to the unique virological profile of Bulgaria. The developed topic is a continuation of the traditions of the National Reference HIV Confirmation Laboratory at the National Center for Infectious and Parasitic Diseases.

The PhD thesis is structured according to the accepted requirements, presented on 203 pages and contains 42 figures and 19 tables. The bibliography includes 198 literary sources. **The overall layout and the evidentiary material are of very high quality and convincingly testify to the professional experience of the doctoral student.**

## **1. Literature review on the topic**

The literature review presents a competent synthesis of information on the topic of the dissertation. Referring to current sources, the PhD student presents the organization, genetic characteristics and life cycle of HIV-1. Special attention is paid to the diagnosis of infection and virological monitoring. This is relevant to the performance of phylogenetic analyses of HIV-1 subtypes in Bulgaria in order to track their spread and evolution. The volume of literature used and its detailed analysis are impressive. Lyubomira Grigorova analyses in detail the works of Bulgarian researchers, which are a valuable contribution to the field of HIV-1 epidemiology. **Summarizing the data on the topic, the doctoral student outlines the importance of phylogenetic studies in relation to many aspects of the epidemic. The response of the immune system to the applied therapies, the possible resistance to certain drugs, as well as the development of vaccines are also of essential importance.**

## 2. Aim of the study and objectives

In relation to the set goal formulated as "to analyse the transmission phylogenetic clusters of the different HIV-1 subtypes introduced and distributed in Bulgaria, through sequencing and phylogenetic analysis of a fragment of the HIV-1 pol gene.", the doctoral student sets himself five tasks. In summary, they include genetic analysis of the HIV-1 pol gene in selected samples and detailed bioinformatic processing of the sequence signatures both in the direction of viral variability and in relation to the dynamics of spread and epidemiological characteristics in the country.

## 3. Materials and methodological approach

The selection of biological material is well thought out and is divided into three groups according to the established subtypes. All necessary conditions have been met, including informed consents, questionnaires and information materials, guaranteeing the requirements of ethical requirements in national and international legislation. **The author describes in the smallest detail the pre-analytical and analytical part of the laboratory tests, which is shown by his professional experience in conducting experimental activities.** In this part, the dissertation work can serve as a methodological guide. The laboratory tests include a set of serological and molecular biological tests conducted during the diagnostic process and virological monitoring of HIV patients in Bulgaria. **The essential part - bioinformatics analysis of the generated sequences, was performed personally by the doctoral student with established software programs. Their application is described competently and with reason.**

## 4. Results and Discussion

The PhD thesis of Lyubomira Grigorova is distinguished by the detailed analyses of the obtained results and their competent discussion in the context of the planned tasks. The separate groups of patients are the following: infected with HIV-1 subtype B, HIV-1 CRF01\_AE subtype and those meeting the criterion of naivety to ART (lack of previous treatment with antiretroviral drugs, which is important for the study of the epidemiology of new cases of infection). The demographic and epidemiological characteristics of patients with the respective subtypes were assessed based on data collected during the diagnostic process. **The obtained results present the specific differences between the studied groups, on which the doctoral student makes a number of comparisons with the literature sources and gives reasoned**

conclusions. The dynamics of demographic and population processes during the study period are commented on. Subtyping and recombinant analysis also provide important information about the spread and molecular characteristics of the infection. The accuracy in building the phylogenetic trees was achieved using modern bioinformatics programs, with several tools used for data processing. **The PhD student draws attention to the fact that in this way not only standard subtypes are identified, but also recombinant forms, which is important for epidemiology.** Summarizing the data for the three studied groups, it can be said that the typical profile of a seropositive patient in our country is - a male person between 20 and 40 years of age from the city of Sofia, who is in a sexual relationship with other men. The data from the PhD thesis should not be ignored, that there are other vulnerable groups that need to be monitored.

## 5. Comments

**This section of Lyubomira Grigorova's dissertation synthesizes the main characteristics of HIV-1 infection in Bulgaria. The retrospective view based on the molecular profile of the virus shows the unique connections between social, cultural and health factors during the period. Vulnerable groups are clearly distinguishable, which allows adequate decisions to be made for the design of preventive programs. The phylogenetic analysis of transmission clusters identified 52 clusters, which indicate the main route of HIV-1 spread in the country.**

## Conclusions

**The PhD student presents six conclusions that correspond precisely to the tasks set. I believe that they synthesize the most significant contributions of the dissertation work.**

## **Final statement**

**This PhD thesis presents a very high-quality study related to the molecular characteristics of common HIV-1 subtypes in Bulgaria. It impresses with the excellent organization of the research work and precise execution and presents the doctoral student as an established specialist in the field of virology, genetics and molecular biology. I cannot fail to note the competent and holistic view of the research subject, which is of essential importance for teamwork with medical specialists. I accept the contributions described in the PhD thesis and believe that they are entirely the author's personal work.**

**I give a high positive assessment of the author's overall scientific and research output and I strongly recommend that the scientific jury award the scientific degree "Doctor" to Lyubomira Svilenova Grigorova.**

Member of the Scientific Jury

(Prof. Dr. Alexey Savov)