



**ATTN. Members of the scientific jury,
appointed by Order No. 196/ 30.06.2025
issued by the Director of the National Center
of Infectious and Parasitic Diseases, Sofia**

OPINION

RE: defense of a dissertation titled: Molecular-virological analysis of the transmission clusters of introduced and spread HIV-1 subtypes in Bulgaria", Supervisor: Assoc. Prof. Ivo Alexiev, Ph.D.

Author: Lubomira Svilenova Grigorova , full-time Ph.D student in the Natl. Lab. For Confirmation of HIV infection

BY: Prof. DSc. MD Radka Mladenova Argirova, Virologist, Clinical Laboratory, Tokuda University Hospital, Sofia

For the award of the educational and scientific degree "Doctor" in the professional field of higher education: 4. Natural sciences, mathematics and informatics; Professional speciality 4.3. Biological Science (Virology – molecular virology ;

DEAR MEMBERS OF THE SCIENTIFIC JURY,

The dissertation presented to me for opinion is a continuation of long years of work on molecular virological and epidemiological analysis and characterization of the HIV-1 epidemic in Bulgaria.

Lubomira Grigorova's current work uses and builds on past research that has applied the rich capabilities of most of the known bioinformatics methods. By their very nature, in this work, the conclusions from the applied research are important and necessary at the population level, as they directly show the course of the HIV-1 epidemic in Bulgaria - the spread of the virus - geographically and among individual communities, target groups and the interaction between them. The data obtained are the basis and oblige for specifically targeted interventions by governmental and non-governmental organizations responsible for public health. On the other hand, molecular virological analyses enrich our knowledge about the evolution of HIV-1, the emergence and spread of resistance – facts

that are important both for the individual and for modern therapy. With these words, I express my conviction in the relevance of the present work.

The dissertation is very rich – a total of 1986 sequences of a fragment of the *pol* gene were analyzed in the laboratory. The sequences included in groups 1 and 2 differ in that the individuals in them are infected with subtype B or with the circulating recombinant form CRF01_AE, respectively. The third group, however, includes individuals naive to therapy – 1053 sequences. This raises the question of the “purity” of this group, since the individuals in it were diagnosed in the period 2012 - 2020, i.e. a period of ART with the constant presence of RT and PR inhibitors, and the majority of these patients - 851 (82.6%) declared that they were infected in Bulgaria. This raises the question of whether there is any data on pre-drug resistance among them. Since the candidate is a co-author of a report analyzing transmissible drug resistance and phylogenetic clusters in Bulgaria, it is interesting to know the quantitative characteristics of transmissible drug resistance in the country and whether it changes during the course of the epidemic. This would also provide an answer to the question of the "purity" of this group.

The goal and tasks are clearly set, their definition shows continuity in the work of the laboratory, the latter to a large extent also evident in conclusions 1,2,3 and 4, which are only partially an expression of personal activity. As for the contributions, contribution N2 is known from previous research of the laboratory and I cannot accept it as a contribution at all. However, the detailed description of the methods used with their advantages and disadvantages suggests that the internet-based tools are well mastered by the candidate.

After successful subtyping of the viral sequences, the analysis of transmission clusters follows. These are groups of viral isolates that show genetic proximity, suggesting that the isolates belong to patients infected probably through common routes. The main objective of this phylogenetic analysis is to establish the genetic relationship between viral isolates from different patients and to identify the routes of virus transmission within certain risk groups. The candidate successfully coped with this task, and moreover - the "archive" data obtained before her and used here allow for a number of comparisons to be made about the course of the epidemic over time - e.g. about the path of entry (introduction) of the virus into the country. The data show that at the beginning of the epidemic, the sources of the virus were mainly in the country, and not infected from outside - 88.4% of the individuals in the first group declared infection in the country. This conclusion contradicts claims from the be The characteristics of the individual clusters and the spread of the virus within them

are comprehensive, informative and confirmatory. The main conclusion of this work is that we must seriously engage in promotional-preventive and information-educational contemporary activities in the new generation of MSM and IDU communities. In this sense, the dissertation work has a real contribution, which is supported by the three published articles (two of them with IF>5), as well as the participation of the candidate in 20 national and international forums. However, greater personal initiative by the author is expected in terms of setting new scientific goals in future publications.

The dissertation is well written and illustrated with 19 tables and 42 figures. My remarks do not diminish the importance and contributions of this work and are more recommendatory and concerning the scientific supervisor.

CONCLUSION: Based on the significance of the results of Lubomira Grigorova's dissertation, I conclude that the candidate has mastered the basic methods and tools of bioinformatics on an HIV model, and is able to interpret the molecular virological data from her research at the population level. During her full-time doctoral studies, she has acquired 495 credit points, has a solid publication record, and her dissertation offers promise not only in the field of HIV, but also in research of other infectious agents with a high mutation rate. All of this gives me a good reason to vote "for" and recommend to the members of the scientific jury to vote "for" awarding the educational and scientific degree "doctor" to Lubomira Svilenova Grigorova in the field of higher education: 4. Natural Sciences, Mathematics and Informatics; Professional field: 4.3. Biological Sciences, speciality “Virology|” (Virology - Molecular Virology).

Sofia, July 12th, 2025

Author: Prof. R.Argirova