To
The Scientific Jury,
Determined by Order No. RD38-404/13.07.2022
of the Rector of SU "St. Kliment Ohridski"
Prof. Dr. Anastas Gerdzhikov

OPINION

by Assoc. Prof. Ivaylo Alexiev Ivanov PhD,

Head of the National Reference Confirmatory Laboratory for HIV, National Center of Infectious and Parasitic Diseases, Sofia

regarding

Dissertation work for awarding the educational and scientific degree of Doctor of Philosophy

of **Venelin Ventsislavov Tsvetkov**,

on topic

"Investigating the impact of physical and biological factors on the realization of herpes viruses"

On the professional direction 4.3. Biological Sciences (Virology)

Scientific supervisor: **Prof. Stoyan Angelov Shishkov PhD**.

I declare that I have no publications in common with the doctoral student Venelin Ventsislavov Tsvetkov and I have no conflict of interest of any other nature within the meaning of Art. 4. para. 4 of ZRASRB. The materials presented to me for the defense of the dissertation work meet the requirements of the ZRASRB and the Regulations for its implementation at Sofia University "St. Kliment Ohridski".

Brief biographical data.
Venelin Ventsislavov Tsvetkov was born in 1993 in Pleven. In 2012, he graduated from secondary education in Pleven. In 2016, he graduated with a bachelor's degree, majoring in "Molecular Biology" and in 2018, he graduated with a master's degree in Virology at the Faculty of Biology of SU "St. Kliment Ohridski".

In 2016, he worked as an office assistant in Pleven, and in 2017-2019, he worked as a biologist at SMDL Ramus Sofia. From 2019, until now, he has worked on clinical studies at Clinpace and Novartis in Sofia.

On 11.07.2018, by order of the Rector of the SU "St. Kliment Ohridski", he was enrolled as a full-time doctoral student in the doctoral program "Virusology" for the acquisition of the educational and scientific degree Doctor of Philosophy, starting from 15.07.2018.

Relevance and significance of the developed topic.
Viruses of the family *Herpesviridae* are some of the most abundant viruses in nature, which can infect a wide range of hosts, including mammals, birds, reptiles, amphibians, fish, and molluscs.

Human herpes viruses have been known since ancient times and cause a wide range of diseases, including latent infections, mild self-limiting diseases, severe generalized life-threatening infections, and carcinomas. It is characteristic of the representatives of the family that they are well adapted to the host and successfully evade the protection of the immune system.

Humans are the only reservoir for Human alpha herpes virus type 1 and type 2 (HHV-1 and HHV-2). The seroprevalence of HHV type 1 is over 90%, and the primary infection with it occurs most often in early childhood. The approved anti-herpes drugs often do not provide the desired definitive treatment results, and vaccines do not lead to the generation of protective immunity. An alternative to medical treatment and vaccines is the use of medicinal plants, they have been known since ancient times and to this day are an additional means in the fight against herpes diseases, and it is to them that the doctoral student Venelin Ventsislavov Tsvetkov is interested.

The dissertation submitted to me for opinion is a successful continuation of the rich history of the laboratory of Virology at the Faculty of Biology of SU "St. Kliment Ohridski", in the development of scientific topics in the field of antiviral activity of various natural sources.

The literature review is developed on 42 pages and occupies about 30% of the volume of the entire dissertation, it contains informative figures and tables that complement the textual explanations. The review presents a wealth of information on the classification of herpes viruses in humans. The morphology and structure of the complex herpesviruses, the localization of the viruses in susceptible cells, and the replication cycle are described in detail. Spatial and up-to-date information on the latent development of infection, the specificities of the immune response, and the pathogenesis of HHV-1 and HHV-2 are provided.

As there is no established approach for universal definitive treatment of HHV, and regarding the topic of the dissertation, special extended attention is paid to the therapy and prevention of HHV, which are presented in detail. Immunotherapy approaches are reviewed, including preparations containing specific antibodies and vaccines, inhibitors of the viral replicative cycle, including nucleoside and nucleotide analogs, and antiherpetic antibiotics. A special place is devoted to phytotherapy, the characteristics, and application of plasma in medicine, and the factors for reducing the transmission of viral infections. This testifies to the good preparation of the student, both in the selection of the studied objects and the arguments in solving the tasks, as well as in the selection of the chosen strategy for carrying out the research.

The objectives of the dissertation were to investigate the effect of plant extracts treated with low-temperature unbalanced gas discharge plasma on the replication and extracellular virions of human herpesviruses and to determine the degree of protection of personal protective equipment. The tasks to achieve the goals correspond to the goals set.

The **Materials and Methods section** is written on 16 pages and is divided into 3 main sections, in which separate lines of research are described to achieve the set goals. This further testifies to the professional skills of the doctoral student in the process of organizing and conducting various experiments of high complexity, such as experiments with cell cultures and the innovative approach to plasma application.

Dissertation results are presented in 30 pages with numerous figures and tables that illustrate the achievements of the doctoral student's experimental work. The obtained results are presented in detail and competently and cover the set tasks. The experimental work includes the relevant control studies characteristic of cell culture studies.

The conclusions show that sufficient convincing data have been obtained to support the set goals and objectives.

In conclusion, I give a positive assessment of the dissertation work and believe that Venelin Ventsislavov Tsvetkov has all the necessary qualities to the awarding of the educational and scientific degree of Doctor of Philosophy.

Date: 05/09/2019 Author of the opinion:

Assoc. Prof. Ivaylo Alexiev Ivanov PhD