STATEMENT

By Prof. Dr. Hristo Petrov Dobrev, PhD, DMSc
Head of the Department of Dermatology and Venereology,
Medical University – Plovdiv,
Head of the Clinic for Skin and Venereal Diseases,
UMBAL "St. George" - Plovdiv

Of a dissertation for awarding the educational and scientific degree **"Doctor"**,
Field of higher education 7. Healthcare and sport,
Professional direction 7.1. Medicine,
Doctoral program "Dermatology and Venereology"

Author: Dr. Gergana Zaprinova Vazheva
Form of doctoral study: Independent preparation
Department: Dermatology and Venereology, Medical University - Plovdiv

Topic: **Application of the PCR method in the modern diagnostics of fungal infections caused by dermatophytes**

Research supervisor: Assoc. Prof. Lilia Alexandrova-Zisova, PhD
Department of Dermatology and Venereology, Medical University - Plovdiv

1. General presentation of the procedure

The set of materials presented to me on an electronic medium is in accordance with the procedure for acquiring the educational and scientific degree "Doctor" and the scientific degree "Doctor of Sciences" at MU - Plovdiv, described in the Regulations for Academic Development at MU - Plovdiv from 2021, and includes the following documents:

- Dissertation
- Abstract
- List and copies of scientific publications and communications on the topic of the dissertation
- Protocol from the Department Council on internal dissertation defense.

The doctoral candidate has attached 3 publications.

The documents meet the requirements for the implementation of the dissertation defense procedure.

2. Brief biographical data for the doctoral candidate

Dr. Gergana Zaprinova Vazheva was born on September 10, 1992, in the city of Plovdiv. In 2017, he graduated from the Medical University of Plovdiv, majoring in medicine. In the period from March 2018 to April 2022, he conducted a regular specialization in skin and venereal diseases at the Clinic for Skin and Venereal Diseases, UMBAL "St. Georgi" - Plovdiv and Department of Dermatology and Venereology, MU - Plovdiv. In December 2022, he acquired a medical specialty in "skin and venereal diseases".

Since March 1, 2019, she has been enrolled as a full-time doctoral student at the Department of Dermatology and Venereology. From 25.02.2020, after winning a competition, she was appointed as an assistant in dermatology and venereology at the Department of the same name at the MU - Plovdiv. So far, he is the co-author of 3 full-text articles. She participated in scientific forums at home and abroad, at some of which she presented clinical cases and posters - Sofia Dermatology Days 2018, 2019, and 2021, 29th EADV Virtual Congress, 2020, and 17th EADV Spring Symposium, 2022. She participated in an intra-university interdepartmental research project. He is a member of BLS, BDD, and EADV.

3. Relevance of the topic

The dissertation is dedicated to the application of a new modern methodology for the diagnosis of fungal infections of the skin, hair, and nails, namely the polymerase chain reaction technique in real-time (real-time PCR).

The topic of the dissertation is current and original because the mentioned methodology is used for the first time in our country.

The dissertation study also has practical value, as the acquired information will contribute to the improvement of the diagnosis of mycotic infections.

The tasks set are appropriate and related to the purpose of the dissertation - to prove the usefulness and importance of the real-time PCR technique for the diagnosis of dermatophyte infections.

4. Knowing the problem

It is clear from the extensive literature review that the doctoral student knows the problem to a sufficient extent.

5. Research methodology

In his dissertation study, the doctoral student used clinical methods – history and clinical examination, and laboratory methods – conventional (direct microscopic examination, cultural examination, macroscopic and microscopic identification of cultures, species identification of Candida yeast, histological examination) and innovative methods (real-time PCR). The methods used are suitable for achieving the goal and obtaining an adequate answer to the tasks set to be solved in the dissertation work.

6. Characterization and evaluation of the dissertation work

The dissertation submitted for review is printed on a total of 142 standard A4 pages. It contains the following sections: contents (2 pages), abbreviations used (2 pages), introduction (2 pages), literature review (35 pages), aim and objectives (1 page), material and methods (18 pages), results (31 pages), discussion (23 pages), conclusion (1 page), conclusions (1 page), contributions (2 pages), bibliography (20 pages), list of publications and participations in the subject (1 page) and acknowledgments (2 pages). It is illustrated with 28 tables and 46 figures, incl. clinical photos.

The content corresponds to the main text. Abbreviations used are correctly reflected.

The introduction and overview are written in a sufficient volume, which shows that the dissertation student has a good awareness of the problem is developed. The overview includes several sections - a general part, which presents detailed information about dermatophytes, a second part, which describes the infections caused by them, a third part, devoted to the methods of examination and diagnosis of dermatophyte infections, and a fourth part, in which describes the polymerase chain reaction (PCR) technique and its application to the diagnosis of dermatophytes.

The purpose of the dissertation work and related tasks are clearly and specifically defined.

A total of 196 patients who passed through the Skin and Venereal Diseases Clinic over a period of 3 years with clinical symptoms suggestive of a fungal infection affecting the scalp, beard, skin or nails were included in the studies related to the dissertation work. Of these, the largest number (n=144) were patients with suspected onychomycosis, and they were further divided into two subgroups – those with diabetes (n=51) and those without diabetes (n=93). All patients underwent history taking and clinical examination, direct microscopic and cultural examination, and Real-time PCR examination. In cases of isolation of Candida yeasts, their species identification was carried out. In 86 patients with onychomycosis, a histological examination with PAS staining of the sampled material was also performed.

Own studies are presented sequentially, described comprehensively and illustrated with a sufficient number of tables, figures and clinical photos.

In the first study, a summary analysis of the results of conventional research methods and the real-time PCR technique for all 196 patients examined is presented. In the following three studies, the results are presented by groups of patients with different localization of the dermatophyte infection - respectively on the scalp and beard, on the skin, and on the nails. In the last fifth study, the results of patients with onychomycosis according to the presence or absence of accompanying diabetes mellitus are presented.

It was found that, in all studied localizations, the real-time PCR technique has a higher diagnostic sensitivity and efficiency compared to direct and cultural examination, while its specificity is comparable to that of cultural examination. Regarding the nails, the results obtained with real-time PCR are similar to those of the histological examination, and when combining the two methods, almost maximum results are obtained in terms of sensitivity, specificity and efficiency of the diagnosis. In patients with and without diabetes mellitus, no significant difference was found between the etiological agents of onychomycosis in the two groups.

In the "Discussion" section, the dissertation student makes an additional analysis of the reasons for the observed rare cases with "inconsistency" of the results obtained with the individual research methods.

At the end of the presentation, 9 conclusions were drawn.

The book collection contains 172 literary sources, of which 10 are in Cyrillic and 162 are in Latin. 31 (18%) of the sources were printed after 2018.

The dissertation is written in correct and easy-to-read Bulgarian language.

7. Contributions and significance of the development of science and practice

The dissertation student has defined a total of 9 contributions, which are distributed as follows: 2 original, 5 original for Bulgaria, and 2 contributions of a scientific-applied nature. All contributions are welcome.

8. Evaluation of publications on the dissertation work

The list of scientific works related to the dissertation includes 3 real publications in national journals, 2 of which are referenced in Scopus, and 4 online participations with electronic posters at scientific forums abroad. In 2 of the publications and 3 of the posters, the dissertation student is the first author.

The number of real publications presented meets the required quantitative criteria laid down in the Regulations for Academic Development of MU - Plovdiv.

9. Personal participation of the doctoral student

The doctoral student was personally involved in conducting the research related to the dissertation work and their summarization and analysis.

10. Abstract

The abstract has a total volume of 67 pages, of which 44 pages are text and 18 pages contain figures, tables and clinical images. The abstract summarizes the material included in the thesis project.

11. Critical remarks and recommendations

The following remarks may be made to the dissertation submitted for review:

1. In the "Materials and Methods" section, the definitions and the methods of calculation and interpretation of the indicators "sensitivity", "specificity" and "efficiency" are not described.

2. In the "Bibliography" section, the Cyrillic references are listed at the end, but should be at the beginning of the bibliography.

CONCLUSION

The presented dissertation contains scientific and applied results that represent an original contribution and meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations for Academic Development of the MU - Plovdiv.

The dissertation shows that the doctoral student Dr. Gergana Zaprinova Vazheva possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Dermatology and Venereology", demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I give my ***positive assessment*** of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions and I propose to the honorable scientific jury to award the educational and scientific degree "Doctor" to Dr. Gergana Zaprinova Vazheva in a doctoral program in "Dermatology and Venereology".

Заличено на основание
Чл.5 §1, б."В" Регламент (ЕС)2016/679

20.03.2023 **Reviewer:** ....

Plovdiv (Prof. Dr. Hristo Dobrev, PhD, DMSc)