

## STATEMENT OF OPINION

by Prof. Tanya Vasileva Strateva, MD, PhD – Department of Medical Microbiology,  
Faculty of Medicine, Medical University of Sofia

*(Member of the Scientific Jury, appointed by Order No. P-2855 / 14.11.2022 of the Rector of the  
Medical University of Plovdiv)*

on a PhD thesis for awarding the educational and scientific degree “**doctor**”

Professional direction: **4.3. Biological sciences**

Field of higher education: **4. Natural sciences, mathematics and informatics**

Doctoral program in **Microbiology**

**Author:** Gergana Boteva Lengerova, MD

**Form of doctoral studies:** independent study

**Department** of Medical Microbiology and Immunology "Prof. Dr. Elissay Yanev"

**Topic:** Evaluation of modern microbiological techniques for rapid identification of microorganisms in patients with bacteremia and fungemia.

**Scientific supervisor:** Assoc. Prof. Michael Mihaylov Petrov, MD, PhD – Department of Medical Microbiology and Immunology "Prof. Dr. Elissay Yanev", Faculty of Pharmacy, Medical University of Plovdiv

### **1. General presentation of the procedure and the PhD student**

The presented set of materials on electronic media is in accordance with the requirements specified in Art. 70 (1) of Section I. Acquisition of educational and scientific degree "Doctor" and scientific degree "Doctor of Science" at MU-Plovdiv; Regulations of MU-Plovdiv from 28.01.2021 and includes all necessary documents.

The PhD candidate has submitted four full-text publications.

I have no critical remarks to the documents.

Dr. Gergana Lengerova graduated with honors from the Medical University of Plovdiv in 2016, where she obtained a Master degree in Medicine and acquired the professional qualification “Physician”. Since 2017, after when she won a contest, she has been an assistant professor at the Department of Microbiology and Immunology “Prof. Dr. Elissay Yanev” at the Faculty of Pharmacy of the MU - Plovdiv, and from 01.2020 – a resident in Clinical Microbiology at the Laboratory of Microbiology at University Hospital “St. George”. During the period 2017–2022, she conducted internships in microbiological laboratories in Latvia, Germany, Turkey and Portugal, under the ERASMUS+ program.

As of 12.07.2021, Dr. Lengerova is an independent study PhD student in the doctoral program “Microbiology” at the Department of Medical Microbiology and Immunology “Prof. Dr. Elissay Yanev”. She is the principal investigator for the Inter-University Project DPDP-5/2018 “Comparative

study on microbiological methods for rapid diagnosis of pathogens from blood cultures” in which a significant part of the experimental work for the dissertation was conducted.

## **2. Relevance of the topic**

In Europe, over 1,200,000 cases of bloodstream infections are registered annually, associated with long-term complications and high mortality, making them a serious threat to public health. An important factor in their control is the rapid diagnosis and the initiation of effective antimicrobial therapy. The traditional approach in the diagnosis of these infections includes clinical and laboratory markers, as well as isolation of the etiological agent from blood culture. In recent years, techniques based on *in situ* hybridization, molecular genetic methods, mass spectrometry, etc. have been developed to optimize the identification of pathogens from positive blood cultures. They are quick and reliable due to their high specificity and sensitivity. Regarding this, I think that the topic of the dissertation work focused on a comparative analysis of the possibilities of three modern methods for rapid microbiological diagnosis of bloodstream infections is extremely relevant and interesting. Currently, similar studies have not been conducted in Bulgaria.

## **3. Knowledge of the problem**

Dr. Lengerova has prepared a literature review on the topic of the thesis based on a large number of literary sources – 375, including 16 in Bulgarian and 359 in English. The number of bibliographic titles from the past decade is 264, which accounts for 70% of all cited sources.

The literature review is very thorough and written with exceptional competence. It demonstrates the author's excellent theoretical education and training. This section contains no superfluous scientific information, only that which relates to the current studies is included. Dr. Lengerova structured the review in 5 subsections as follows: 1. Bloodstream infections (bacteremia, fungemia, septic conditions) – history, epidemiology, definition, and clinical forms; 2. Etiology of bloodstream infections; 3. Antimicrobial drug resistance of isolates from positive blood cultures; 4. Microbiological diagnosis of bloodstream infections; 5. Summary of the literature review. In the summary, the currently unresolved issues regarding the microbiological diagnosis of bloodstream infections in Bulgaria are highlighted, as well as Dr. Lengerova's motivation for developing the current dissertation work. This interesting approach is a logical transition to the “Aim and Objectives” section.

## **4. Research methodology**

The PhD student describes the study design and patient inclusion/exclusion criteria in the current thesis concisely and accurately. Routine methods for microbiological diagnosis, as well as modern methods for identifying the etiological agents of bloodstream infections were used, such as quick *in vitro* diagnosis by multiplex polymerase chain reaction (mPCR) using a FilmArray apparatus; fluorescence *in situ* hybridization (FISH) by QuickFISH BC test; and MALDI-TOF MS analysis (mass spectrometry) performed with a Vitek ® MS apparatus. I am delighted to note that some of the results have been confirmed by the National Center for Infectious and Parasitic Diseases – Sofia, ensuring the accuracy of the PhD candidate's work. The extraordinarily well-executed statistical analysis, for which Dr. Lengerova used specialized software and professional consultation of an expert from the Department of Social Medicine and Public Health, Faculty of Public Health, MU – Plovdiv, deserves attention.

## **5. Characterization and evaluation of the PhD thesis and its contributions**

The dissertation consists of 197 standard typewritten pages and is richly illustrated with 14 tables, 68 figures and 6 appendices with excellent overall quality. The work is well balanced between

its individual parts and structured as follows: Title and introductory pages – 2 pages, List of the used abbreviations and symbols – 2 pages, Content – 3 pages, Introduction – 2 pages, Literature review – 32 pages, Aim and Objectives – 1 page, Materials and Methods – 13 pages, Results – 67 pages, Discussion – 27 pages, Conclusions and contributions – 3 pages, Bibliography – 22 pages, List of publications and scientific reports related to the topic of the thesis – 3 pages, and Appendices – 18 pages.

The aim of the thesis is argumentative. Five objectives have been clearly and precisely formulated for its achievement.

The results are presented sequentially, following the previously set tasks. The discussion is in-depth and includes the studies published so far by Bulgarian and foreign authors. Original results demonstrate the advantages of the methods, chosen by the PhD student, for the etiological diagnosis of bacteremia and fungemia (FISH, mPCR and MALDI-TOF MS) over the conventional ones, widely used in diagnostic microbiological laboratories in the country. Dr. Lengerova recommends using FISH and mPCR in addition to the routine microbiological examination of blood in order to provide earlier proof of the causative agent and an accurate evaluation of the patient's therapy. The high speed, sensitivity, and low cost per sample present MALDI-TOF MS as a potential routine method for microbial identification, especially for Gram-negative bacteria and fungi. In the Plovdiv region during the period 2015–2020, Gram-positive bacteria dominate the etiological structure of bacteremia, whereas *Candida non-albicans* species dominate fungemia. The incidence of methicillin-resistant *Staphylococcus aureus* decreased, while the frequency of vancomycin-resistant enterococci, ESBL- and KPC-producing Gram-negative bacteria increased. Commonly, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are among the leading etiological agents with problematic resistance, including all target antibiotics without colistin.

There have been drawn eight conclusions that are logical, consistent with the results obtained, and in line with the aim and objectives specified.

I accept the nine contributions formulated by the PhD student, which are divided into two categories: original contributions and contributions enriching existing data, and practical scientific contributions. I would like to emphasize the following as being the most significant:

1. A pilot study in Bulgaria has conducted a comparative assessment of the diagnostic value of rapid methods for diagnosing positive blood cultures – FISH, multiplex PCR and MALDI-TOF MS in patients with bloodstream infections.
2. A comprehensive analysis (a six-year ambispective study) of the dynamics and trends in the etiology and antimicrobial drug resistance of isolates from positive blood cultures in the largest university hospital in the country was performed, and the leading pathogens and types of antimicrobial drug resistance were identified.
3. The first financial analysis of the direct treatment costs of patients in the ICU diagnosed with bacteremia/fungemia during intensive care stay and evaluation of the effectiveness of the applied methods for diagnosing positive blood cultures was performed.
4. A modified algorithm was developed and implemented for the direct identification by MALDI-TOF MS of pathogens from blood cultures, immediately after their positivity.

#### **6. Evaluation of the publications and personal contribution of the PhD candidate**

Dr. Lengerova attaches a list of 4 full-text publications, in three of them, she is the first author. Her active participation with posters and oral presentations in national and international scientific forums makes a great impression, as from 2018 to today she has presented the results of the studies carried out at eleven forums, while she is the first author of most of them. All this is compelling proof of Dr. Lengerova's leading role in the developments made.

## 7. Author's summary

Regarding the structure, content, presentation, and quality of the author's summary, I have no critical comments. It was prepared in accordance with generally accepted standards and reflects the main results achieved in the dissertation.

## CONCLUSION

The dissertation work of Dr. Gergana Lengerova, titled "Evaluation of modern microbiological techniques for rapid identification of microorganisms in patients with bacteremia and fungemia", focuses on a current problem in medical microbiology and makes original and scientifically-applied contributions to the fields of clinical microbiology and hospital epidemiology.

In my opinion, the dissertation corresponds to the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its implementation, and the Regulations of the Medical University of Plovdiv. The doctoral candidate possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Microbiology", demonstrating the qualities and skills necessary for conducting independent scientific research.

Having considered all of the foregoing, I confidently give my *positive assessment* of the conducted scientific research presented in the above-reviewed dissertation and author's summary *and propose to the esteemed scientific jury to award the educational and scientific degree "Doctor"* to Dr. Gergana Boteva Lengerova in a doctoral program in Microbiology.

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

12.12.2022

Statement by: ..........

/Prof. Tanya Strateva, MD, PhD/