

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University

Applicant

Habilitation thesis

Reviewer

Reviewer's home unit, institution

Mgr. Petr Králík, Ph.D.

Use of PCR-based methods in microbiology and their specific applications

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The habilitation thesis by Mgr. Petr Králík, Ph.D., is focused on the development of PCR-based methods in microbiological diagnostics. Dr. Králík has been working in this field for more than 20 years and his long-standing experience is reflected in the submitted thesis. The work is highly relevant, as pathogens, including emerging pathogens, pose a serious problems in human and veterinary medicine, and their reliable diagnostics contributes to both treatment improvements and disease prevention.

The thesis is written on 204 pages and consists of a brief description of the obtained results and a collection of 17 scientific papers (two reviews and 15 experimental papers) published between 2008 and 2022 dedicated to the study of three areas: the utilization of qPCR in quantification of microorganisms, the determination of viability by culture-independent techniques, and advanced multiplex PCR detection using xMAP technology.

A substantial part of the thesis describes applications of PCR methods for detection and quantitation of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease, in veterinary samples. An important result of the thesis was the implementation of qPCR for the precise quantification of MAP in cow faeces which is essential to distinguish truly infected animals from passive shedders. This distinction is crucial for informing management decisions and limiting the spread of MAP within and between herds. The rigorous standardization of used methods was applied as an important part of all presented papers. The main contribution of Dr. Králík was the introduction of a universal reference standard, evaluated by digital PCR technique, that could be used in multiple laboratories and finally it was commercially applied.

Methods based on qPCR with intercalating dyes for determination of bacterial viability were another important question solved in the thesis. Optimized protocol using PMA was shown to be valuable when MAP is detected in milk and it was also used for the study of MAP exposed to antibiotics, disinfection agents and probiotics.

In the third part of the habilitation thesis applications of xMAP technology developed by Luminex Corporation was used for multiplex DNA-based detection of microorganisms. MOL-PCR was selected as the most reproducible and sensitive method due to the use of PCR with universal primers and same length products. In the study Reslova et al. (2018), precise MOL-PCR assay optimization was described including probe design, selection of enzymes and chemicals and reagent concentrations, which was used for detection of ten food-borne pathogens. In following studies the method was expanding for determination of biothreat bacteria and food and water-borne viruses and its parameters were confirmed by collaborative validation. Developed methods can be implemented as a routine diagnostic for the pathogen and biothreat detection in environmental samples.

Based on the overall content, I consider the habilitation thesis to be of high quality and beneficial to the research community. The thesis, in addition to demonstrating Dr. Králík's scientific expertise, also shows his ability to master numerous methods of molecular biology and microbiology, including innovative techniques.

Questions for the habilitation thesis defence

1. What are the main advantages of MOL-PCR compared to MLPA in pathogen detection?
Are there some other alternatives for MOL-PCR product detection?
2. Are there some possibilities to increase the MOL-PCR sensitivity?
3. What is the current epidemiological situation of MAP infections in cattle in the Czech Republic? Is the number of cases decreasing thanks to early diagnostics?

Conclusion

The habilitation thesis entitled "Use of PCR-based methods in microbiology and their specific applications" by Mgr. Petr Králík, Ph.D. **fulfils** requirements expected of a habilitation thesis in the field of Molecular biology and genetics.

Date: 25. 7. 2025

Signature: