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Editorial

Guest editor's introduction for BDQ special issue: 'Advanced Molecular Diagnostics for Biomarker Discovery'

Dear reader,

I have the honour to present the first special issue in 'Biomolecular Detection and Quantification' (BDQ) which is entitled 'Advanced Molecular Diagnostics for Biomarker Discovery'. It is published in conjunction with the last qPCR and NGS 2015 event which took place in March 2015 in Freising Weihenstephan, Germany (www.qPCR-NGS-2015.net) covering the same theme. This aim of this special issue is to present the newest developments in highly sophisticated molecular techniques and related scientific applications in combination with the latest data analysis tools. Furthermore, the implementation of these advanced molecular diagnostics tools for discovery, quantification and validation of molecular biomarkers will be presented. Since 2004 the history of this conference series was primarily focused on quantitative PCR (qPCR) related techniques and applications, but since 2010 the focus was broadened to digital PCR (dPCR), next generation sequencing (NGS) and the underlying complex data analysis applying bioinformatical tools. This history is nicely summarized by the BDQ editors in one of the previous editorials '*qPCR, dPCR, NGS – A journey*' [1].

Today, biomarkers have immense scientific and potential clinical value in the diagnostic testing pipeline. They span the broad diagnostic sector from the genome to the phenome over various '-ome' levels and have been used since the earliest days of the application of molecular biology. A biomarker signature is capable of revealing specific biological traits or measurable physiological changes, according to a disease status, physiological or pathological condition, or after drug application [2]. As novel gene-based diagnostics proliferate, they will be increasingly important to drug development, approval and later in clinical practice. There are numerous promising singular biomarkers or more complex multiple biomarker signatures available, the most important of which are currently used for assessing drug development, patient stratification or measuring the efficacy of treatment in therapeutic medicine. Clearly there is a translation problem to transfer the results from molecular diagnostics research to drug development and finally clinical practice [3,4]. In future, biomarkers and their interaction on various '-ome' levels will increase the molecular and cellular knowledge of disease and drug mechanisms.

The first goal in the biomarker development pipeline is the generation of reliable biological data from applied diagnostic techniques and applications. Therefore, an international consortium of scientists, working in various fields of nucleic acids molecular diagnostics established working guidelines for qPCR and dPCR [5,6]. These, so called, MIQE guidelines have set new standards in clinical, veterinary and agricultural diagnostics science and are highly appreciated by the scientific community, which is evident by the high citation rate of these publications.

An important step in the workflow of molecular diagnostics and biomarker development is driven by tissue limitations and the related methodological considerations. Therefore the first focus of the presented publications is dedicated to nucleic acid degradation and the impact of *RNA quality and RNA integrity to ensure transcriptional biomarker validity* and how *post-mortem and physical degradation affects RNA stability*. A further MIQE compliant publication is focused on the optimization of the qPCR workflow and data analysis by *removal of between-run variation in a multi-plate qPCR experiment*.

Novel classes of biomarkers are circulating nucleic acids which are free floating in blood, bound to proteins or coating cellular micro-vesicles. Most prominent is the microRNA family, which was recently detected as free, extracellular RNA in the bloodstream [7]. Their potential application as circulating biomarkers in 'liquid biopsies' was promptly recognized and investigated, because they are capable of distinguishing diseased individuals from healthy probands. The non-invasive nature of circulating microRNA collection and their sensitivity and specificity in diseases has encouraged intensive microRNA biomarker research. Since then, circulating extracellular small RNAs (smexRNA) have been detected in a variety of body fluids, e.g. milk, saliva, tears, sweat, cerebrospinal fluid, urine, etc. [8]. Herein we present *the potential of smexRNA in veterinary diagnostics to serve as new biomarker signatures* by applying multivariate data analysis.

A further focus in this special issue 'Advanced Molecular Diagnostics for Biomarker Discovery' is dedicated to high throughput genomics and transcriptomics technologies by applying NGS techniques. This advanced technology potentially allows a holistic view and generates numerous candidate biomarkers with potential diagnostic or clinical value, but the practical value of these broadly scattered biomarkers remains uncertain. The implementation of the NGS workflow into the clinic is challenging, due to insufficient control for variation in patient sample loading, target amplification efficiency, library preparation and later sequencing error. One of the publications in this special issue addresses these methodological considerations in the NGS workflow – *control for stochastic sampling*

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variation and the polymerase error. In the recent years, time consuming Sanger sequencing has been increasingly replaced by parallel resequencing of multiple genes using NGS technologies. Molecular diagnostic laboratories or commercial vendors often develop customized NGS resequencing workflows for specific diagnostic portfolios. Herein the application of a new target enrichment strategy is presented, enabling the resequencing of any human genomic region of interest by *targeted resequencing and variant validation using 'pxlence' PCR assays.*

While the quality of the sample to be investigated is an important consideration, sample availability is often limited. Hence, in human diagnostics there is always the challenge to detect numerous biomarkers from limited sample material. Taking this to the extreme, we are able to sample single cells, such as circulating tumor cells (CTCs), and quantify the cellular nucleic acid content. One of the special issue publications focusses on this area of research, exploring the feasibility of a protocol for the *isolation and molecular characterization of single CTCs from cancer patients using a single-cell NGS approach.*

A further source of variance in sample measurement is the introduction of pre-amplification methods to make the essential nucleic acid biomarkers measurable with such limited starting material. This topic is addressed in a contribution on the *evaluation of bias associated with high-multiplex and target-specific pre-amplification.*

To achieve reproducible diagnostic signatures and physiological meaningful answers, various complex bioinformatical tools have to be applied for data analysis. The integrative analysis of multilevel biological markers, most prominent the microRNA–mRNA network, and additionally the analysis of genomic, proteomic, or metabolomic markers will help to understand the molecular interaction between the '-ome' levels, and hence the impact on physiology. By using sophisticated bioinformatic tools to integrate singular biomarker datasets, one can identify regulatory networks on the post-transcriptional level and even beyond [9]. These regulatory networks and the connected biomarkers help to understand the disease processes, the interaction with drugs and will be essential to shape meaningful clinical conclusions. Thus, the use of biomarker signatures and the connected molecular network, and their successful translation into clinical practice are likely to play an important role in developing and promoting personalized medicine in the near future.

I hope the selection of the presented publications in this first BDQ special issue 'Advanced Molecular Diagnostics for Biomarker Discovery' has attracted your attention and will help to solve further analytical challenges in your own biomarker development pipeline. You can watch a selection of recorded talks presented at the qPCR and NGS 2015 symposium in Freising-Weihenstephan via our streaming portal *eConferences – Amplify your knowledge in qPCR, dPCR and NGS!* (eConference.qPCR-NGS-2015.net). This streaming portal is dedicated to scientists from the community of qPCR, dPCR, NGS, and Molecular Diagnostics. You will find all the records from around 280 presentations from various events in the past years (2010–2015). We provide the presentations for FREE via movie streaming technology in high quality, high resolution and perfect sound quality in high speed.

Enjoy reading and watching.

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Guest Editor
Michael W. Pfaffl
Physiology Weihenstephan, Technische Universität München, Weihenstephaner Berg 3, 85354 Freising, Germany
E-mail address: Michael.Pfaffl@wzw.tum.de