Nucleic acid quantification—progress and pitfalls

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Half a century after the Nobel Prize for Physiology or Medicine was jointly attributed to Francis Crick, James D. Watson, and Maurice Wilkins “for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material,” the development and use of accurate methods to quantify nucleic acids in their different forms still remain a challenging exercise.

Too many “scientific” results using inappropriate or incomplete analytical procedures have been published. A “quick and dirty” estimation of the total nucleic acid concentration followed by an uncontrolled extraction step generating a solution enriched in nucleic acid that is partially amplified during a polymerase chain reaction (PCR) or hybridized on a microarray is often providing results that cannot be repeated or are strongly biased.

Despite the increasing use of measurement results for nucleic acid amounts in decision making, may it be in the frame of genetically modified organisms (GMO) trade or in medical diagnostics, until recently more efforts have been directed (e.g., by instrument developers) to increase the sample throughput rather than to improve the quality of analytical data. The lack of reproducibility and comparability of measurement results as well as of standardized procedures represent major hurdles for translating experimental findings into sound generic knowledge and new tools, required for instance in healthcare.

Two kinds of actions seem to be necessary for improving the balance between quantity (amount) and quality of data: on the one hand raising awareness about existing ‘good practice’ guidelines and stimulating their application and on the other hand developing and disseminating new approaches for modern analytical quality assurance and metrology. Both activities should be supported by scientific publishing.

Therefore, this topical collection of *Analytical Bioanalytical Chemistry* is devoted to problems related to the quantification of different nucleic acid molecules. In the following, mRNA used for gene expression measurement by reverse transcription quantification PCR (Sanders et al.), standardization of measuring cell free DNA in plasma (Devonshire et al.), and effects of methylated CpG sites (Burke et al.) are dealt with. Efforts to obtain reliable quantification of GMO (Milavec et al.) and pathogenic bacteria in plants (Dreo et al.) are also discussed.

From a conceptual point of view, nucleic acid quantification is not different from any other chemical measurement. It is indeed a multistep process, starting from adequate sampling, followed by an extraction procedure, quantification itself, and finishing with data analysis and reporting of the results, including an appropriate measurement unit. Uncertainty components are associated with all those steps and they contribute to the variability of the final result.

Sanders et al. have reviewed the different parameters that affect the quantification of mRNA by reverse transcription quantitative PCR. The importance of appropriate RNA isolation and storage conditions, and of the choice of relevant reference genes are discussed, as well as the difficulty in coping with the inherent biological variability of patients. It is also stressed that the upstream process, prior amplification by the polymerase, is creating the major contributions to the variability of results.

The importance of an appropriate extraction protocol during the quantification of nucleic acids in “liquid biopsy” is

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also discussed by Devonshire et al. The accurate quantification of total cell-free DNA will aid future clinical implementation through quality assurance of the technical performance.

Quantification of RNA or DNA by PCR requires the use of calibrants, which should be ideally amplified with the same PCR efficiency as the sample analyzed. The use of appropriate and commutable or at least harmonized calibrants is a common challenge of all methods based on the quantitative PCR principle. Sanders et al. discuss this issue for gene expression quantification, but it is also relevant for GMO quantification as reviewed by Milavec et al.

The unique feature of PCR as analytical principle consists in generating a signal from a very specific nucleic acid sequence. It is also its greatest weakness as the process is based on an enzymatic reaction with its intrinsic limitations. PCR in its so-called digital version overcomes a number of difficulties related to the commutability of calibrants as the method does not rely anymore on external calibration. Not only purified solutions containing either RNA or DNA can be portioned out, but Dreo et al. show here also the relationship between colony-forming units and direct counting of encapsulated pathogenic bacteria by droplet digital PCR.

The benefit of having carefully designed and characterized reference materials to verify the performance of an analytical method is clearly illustrated by Burke et al. They demonstrate the lack of agreement among specialized laboratories measuring the methylation ratio of the human CDKN2A tumor suppressor gene that is associated with a complete loss of expression in many cancer types. The study reveals the weakness of the bisulfite conversion methodology and questions the concept of measuring regional methylation by averaging the methylation ratios of many CpG sites.

As reported in this issue, significant progress has been made to obtain better and more reliable data that are on a par with trustworthy diagnostics and decision making. But continued efforts are still required to achieve full confidence in measurements results.

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