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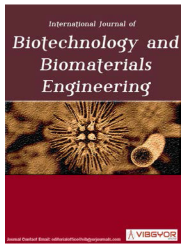
Biometrology: The Relationship between Biotechnology and Metrology, its Historical Background and its Applications



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Tenaglia Giunta Brenda[1,2*] and Napán Rocío[1]

[1]*Departamento de Termodinámica, Instituto Nacional de Tecnología Industrial (INTI), Metrología Física, San Martín, Buenos Aires, Argentina*

[2]*Universidad Nacional de San Martin (UNSAM), San Martin, Buenos Aires, Argentina*

Abstract

This review aims to bring the readers closer to the scientific background on the measurements science and introduce them to a new concept that is gaining popularity among the scientific community: Biometrology. Surely, the readers will wonder what biometrology is and what its applications are. These questions and others will be addressed throughout this paper, citing examples of different applications in other knowledge areas to finally conclude with the presentation of a definition focused on the life sciences.

Keywords

Biometrology, Metrology, Biotechnology, Quantity, Accuracy

Introduction

Since ancient times, human evolution has been linked with measurements. In every aspect of our life, measurements are present, and we are often unaware of how necessary and how valuable they are. Everything around us has been measured or is continuously being measured. Every day we live with the seven basis units and their derivatives that form the International System of Units (SI): Time, length, mass, amount of substance, temperature, electric current and luminous intensity. But since when are things measured? Who assure us that "the meter" which we have at home is the same around the world? Who are the scientists responsible for measurements? For biotechnologists, is it important to be aware of the measurement they make?

Metrology and Biotechnology: State of Arts

Metrology: Measurement and historical overview

What is metrology? According to the ***International Vocabulary of Metrology (VIM)***, metrology is the science of measurement and its applications. Further, *"Metrology includes all theoretical and practical aspects of measurement, whatever the measurement uncertainty and field of application."* [1]. The first records of the act

***Corresponding author:** *Tenaglia Giunta Brenda, Departamento de Termodinámica, Instituto Nacional de Tecnología Industrial (INTI), Metrología Física, San Martín, Buenos Aires, Argentina; Universidad Nacional de San Martin (UNSAM), San Martin, Buenos Aires, Avenida General Paz 5455, San Martin, Buenos Aires, B1650WAB, Argentina, Tel: (5411)-4724-6283*

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of measurement date back to years before the Christian era. Archaeological findings from ancient Chinese and Egyptian civilizations prove the early mastery of metrology. By 1600 b.C, China already had its own decimal metric system, and the perfection of the Egyptian pyramids are a reliable proof of this [2].

Human societies continued to evolve, and the measurement systems were established. In those days, the scientist started to discuss about the necessity to define universal measurement units. The French Revolution was a milestone that changed the metrology forever because the meter and the kilogram were defined and consequently, a decimal metric system (DMS) was introduced [2].

The ***Convention of Meter*** is a diplomatic treaty which was signed by ambassadors of seventeen nations in Paris on May 20th, 1875. The main goal was the unification of DMS and its improvement. At the same moment, the ***Bureau International des Poids et Mesures (BIPM)*** was created. Further, member states were summoned to a meeting in the ***Conférence Générale des Poids et Mesures (CGPM)*** every four years and a ***Comité International des Poids et Mesured (CIPM) with*** members of 18 countries was consolidated. Both CGPM and CIPM are responsible for overseeing the BIPM activities [3]. However, due to the scale of activities assigned to the latter, in 1927 CIPM began to create the ***Consultative Committees (CC).*** Each one specialized in specific particularities of each field of science and composed by international experts [4]. In summary, thanks to the joint works of these organizations we have certainty that "*the meter*" we have at home is the same around the world. And they are constantly working to provide us with measurements that are accurate, precise and with enough degree of confidence.

Around 1961, the SI was almost defined after the 11th CGPM and it was formed by 6 basic units: second, kilogram, meter, kelvin, ampere and candle. The last unit defined was the mole in the 14th CGPM which took place in Paris in 1971. Even though the mole was established, the scientists and members of CIPM of that time did not consider the field of chemical metrology to be relevant. The reasons of that resistance are a question mark because in that moment some organizations had research which reflected the necessity to develop a metrology infrastructure for chemistry [5]. Perhaps, the scientific community considered metrology issues to be only concern to physics and nowadays we can assure it is a science that concerns us all.

It was only in 1990 when CIPM decided to investigate the viability of significant chemical metrology activities. Finally, in 1993 the ***Consultative Committee for Amount of Substance (CCQM)*** was formed by several working groups specialized in different areas of chemistry and biology [5].

The new millennium had already begun and the interest of the scientific community for biotechnology and their application fields was increasing day by day. Recombinant deoxyribonucleic acid technology was evolving exponentially, nevertheless some researchers began to wonder about results credibility. But Who were those scientists? They were the metrologists who are in charge of measurement sciences. With these questions posed, the ***Bio-Analysis Working Group (BAWG)*** was created in 2000. Several year later, BAWG was divided in three new groups in 2014:

- ***Nuclei Acid Working Group (NAWG)***
- ***Protein Analysis Working Group (PAWG)***
- ***Cell Analysis Working Group (CAWG)***

Moreover, due to the importance and the diversity of activities that they were developing, the scope of CCQM was expanded and it was renamed as ***"CCQM-Metrology in Chemistry and Biology"*** [5].

Until then, it could be thought that the vitally important changes in metrology had already happened. However, another historical event was about to happen: the redefinition of units and, among then, the mole. During the last decade, technological advances have made it possible to redefine almost all basic units of SI in terms of fundamental constants of nature [5]. It was at the 26th CGPM in 2018 when it was proposed that the mole should be defined by setting the value of the Avogadro's constant. Finally, on May 20th, 2019, the redefinition of SI, and more precisely of mole, was a fact. Today, the new definition is the following:

"One mole contains exactly $6.022\ 140\ 76 \times 10^{23}$ elementary entities. This number is the fixed numerical value of the Avogadro constant, N_A, when expressed in the unit mol^{-1} and is called the Avogadro number" [6].

Metrology in Biotechnology: Is it feasible nowadays?

More profound knowledge**s** in genetics and molecular biology enabled intellectuals of the second half of the 20th century to develop genetic engineering techniques, thereby generating genetically modified organisms (GMOs). All of them are at the heart of contemporary biotechnology in which its most outstanding activities include isolation, identification, manipulation, storage and gene creation [7]. However, none of this would have been possible without one of the most important discoveries in 1953, when Watson and Crick elucidated the double helical structure of DNA. This discovery was key in the development of recombinant DNA technologies in 1973, the improvement of the polymerase chain reaction (PCR) presented by Mullis in 1984, the cloning of Dolly sheep in 1996, until reaching the entire collection of available techniques used by scientists around the world. At the moment, it could be said that it was utopian to think that metrology is part of biotechnology. Even for these illustrators, with their revolutionary findings, perhaps thinking about it was unimaginable. However, without the contribution of metrology none of these momentous facts would have been possible. Now, at the same time but in different places, are we aware that, in our routines, in the laboratory, in the equipment, almost invisible, metrology is present? Are we aware of the traceability of the results? Do we check if the method that we are using has been verified?

By 1995, some molecular biologists were already beginning to become aware of the importance of metrology in its fields of application. During the twentieth CGPM, biochemist Mariane Grunberg-Manago gave a speech on the importance of measurements in her work. There she briefly described human genetics and how it has been possible to identify some genes by mapping the human genome responsible for genetic diseases. Faced with a community that may have never imagined the possibility of measuring DNA fragments, she ended the speech with the following words:

"I have tried to show you how molecular biology needs the discipline of metrology to which you yourselves are attached. You will, during this week, refine this art which is yours" [8].

This indicated the birth of what we might call biometrology today. A new science in which two disciplines began to merge and metrologists became aware that their work went even beyond physics and chemistry.

A new CGPM was convened in 1999, the twenty-first. There, it was established that the significant growth of biotechnology in health, food production, forensic medicine and environmental protection entails the need for accurate measurements traceable to SI and encourages to have an adequate metrological system [9]. Finally, at the sixth meeting of the CCQM, the formation of the *ad-hoc* bioanalysis working group was achieved under the conduction of Helen Parkes [10]. The promoters of the creation of this group proposed to develop and maintain a structure of critical measurements and standards to support the biotechnology industry. In 2003, several common biomed problems were raised in the context of gene, protein and cell analysis. As the first line of work, they selected DNA quantification for the purposes of improving the comparability of these measurements and determining the factors that contribute to an accurate and precise result with their corresponding uncertainty value. The first study focused on quantitative PCR (qPCR) and then followed by others, including: isotopic dilution mass spectroscopy (IDMS) for DNA quantification, quantification of peptides and proteins by mass spectrometry, fluorescence in ELISA (Enzyme-Linked Immuno Sorbent Assay), etc [11].

Since the mid-1990s, in which the debate on metrology in biosciences was opened, scientists have developed their projects seeking to solve those metrological problems that no one had previously focused on. They have worked on within what we can now call "Biometrology". Later we will focus on the definition of biometrology, meanwhile it is important to note that the CAWG, PAWG and NAWG are currently working for the purpose of:

"Developing a biometrological infrastructure that allows to obtain increasingly accurate, precise, reproducible and traceable results to SI".

Will it be possible to consider metrology in biotechnology? Is it a reality or is it still part of an illusion? Everything that seemed to be utopian in the last century, in which very few people were aware of the importance of metrology in biosciences, today is a reality, a fact and it is motivating to discover day by day all that remains to work on.

Biometrology and its beginnings

It would be absolutely right to attribute the beginnings of biometrology to the work carried out by Helen Parkes who**,** by the late 1990s, had already questioned the need for a metrological structure in measurements applied to life sciences [12]. On several occasions, she already made clear that the lack of reproducibility of the measurements was an issue that could not pass unnoticed.

In 1995, she participated in a project intended to quantify oligonucleotides through DNA-DNA hybridization. The idea was to use optical biosensors that would allow quantification in real-time, fast and that the biosensor can be reusable [13].

During those years, diverse scientists developed different methods that allowed the rapid extraction of DNA from different samples. Many of them are widely used today but, which is the method that allows to obtain a complete sample and with the highest degree of purity. In 1996, H. Parkes and other members of the working group raised the same question. As a result, they compared six methods for rapid DNA extraction into plant tissues and rated them according to their yields [14].

The identification of different species of meat in raw and cooked products using oligonucleotide probes was another work in which H. Parkes participated in 1997. These probes were species-specific and allowed, for example, the detection of products of adulterated meat through a hybridization assay and semi-quantification [15].

With the advancement of biotechnology for application in the field of medicine, the accuracy and precision of the results were increasingly demanding. Let us remember that by 2000 during the sixth meeting of CCQM, H. Parkes had demonstrated the relevance of the problems that arose when it came to measurements in biological systems. The corollary of that meeting was the consolidation of the BAWG [12].

In 2001, the United Kingdom launched a program called "Measurements in Biotechnology". One of the initiatives was based on the quantification of DNA by qPCR. With their collaborators, H. Parkes demonstrated that the combination of the PCR and MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization - Time of Flight) technique presented promising results. In proteomics they raised the mass spectrometry coupled to chromatography, as a primary reference method. Finally, at the cellular level, they suggested the need for methods that would allow the authentication of cell lines that were used in research work. With this program, the discussion was opened up to the bioscience community on how it is best to apply metrological concepts, such as traceability and uncertainty, and demonstrate the benefits of comparable measurements [16].

In 2003, H. Parkes along with BAWG members, began qPCR pilot studies coordinated by the Laboratory of Government Chemistry (LGC) and the National Institute of Standards and Technology (NIST). The purpose was to determine the factors that contributed to interlaboratory variability in DNA quantification. The results showed good repeatability but were not reproducible. In 2004, research continued, and it was proposed to achieve more effective identification of variables that contributed to reproducibility [12].

From 2007 to 2010, she participated in the first key comparison made by the BAWG. The objective of this work was to support calibration and measurement capabilities (CMC) for the determination of plasmid DNA in an aqueous solution [17].

"Traceability of complex biomolecules and biomarkers in diagnostics-effecting measurement comparability in clinical medicine", was a project that H. Parkes coordinated between 2008-2010. The scope of this was the development of measurement methods for two biomarkers: human growth hormone (hGH) and C-reactive protein (CRP) that provide traceable results to SI [18].

In 2011 she published, together with other researchers, a final report studying measurement services and comparison needs in an international infrastructure for biosciences and biotechnology. The idea was to provide contributions to the future BIPM program, as well as being a useful reference for the ***National Metrology Institutes (NMI).*** In addition, the study focused on measurements of proteins and nucleic acids for the health sector because these were the areas of greatest interest for BIPM [19].

Between 2012 and 2015, H. Parkes contributed to the team working on *"Metrology for the monitoring of infectious diseases, antimicrobial resistance and harmful microorganisms".* Highly accurate methods with traceability to SI and materials were developed to allow the quantification of infectious disease-causing pathogens in clinical samples [20]. Simultaneously, between 2013 and 2016 she coordinated *"Traceability for biological molecules and relevant entities"* where methods and protocols were designed using purified reference materials and relevant biological molecule enumeration techniques such as nucleic acids, proteins and cellular entities were developed [21].

The evaluation of digital PCR (dPCR) as a primary reference measurement method to support advances in precision medicine was published in 2018. dPCR was validated as an SI traceable measurement process and demonstrated how it can be used to determine the number of DNA copies of reference materials that were in aqueous solution [22].

In 2019, H. Parkes, et al. produced a document whose focus was based on the requirements necessary to ensure quality in patient samples in the context of the new in-vitro European diagnostic regulation that will take effect in 2022. They developed pre-analytic process protocols for quality assurance in the collection and handling of samples and in consequence in the determination of medical diagnosis [23].

Today, H. Parkes continues to develop its biometrological activities in LGC laboratories, bringing new knowledge to the scientific community and inspiring new generations to continue working towards a common aspiration: Biometrology.

Biometrology: A Multidisciplinary Science

The concept of biometrology was mentioned briefly in the previous paragraphs. In short, we could define it *as metrology applied to life sciences.* However, we can infer that this word applies in different areas of knowledge. There are a variety of topics that seem unrelated to the naked eye, but a common word is clearly identified: *Quantify,* according to the works of H. Parkes. Examples are listed below. It is important to note that the list is endless, and we will only focus on a few, encouraging the readers to continue searching for them.

Biometrology in medical sciences

Early diagnosis of neurodegenerative diseases is limited by the lack of non-invasive methods to make it like Alzheimer's and Parkinson's. Since 2016, a multidisciplinary group, including H. Parkes, have been working to overcome the limitations of biomarker measurement methods for diagnosis and treatment. They have made very promising progress and are still investigating in order to develop protocols, reference methods and standards to quantify biomarkers with metrological traceability to SI and with a low level of uncertainty [24].

Biometrology in food

In the field of food chemistry, biometrology is becoming increasingly important due to consumer demands and regulatory bodies on food labelling, especially those derived from biotechnology with the detail of its components [25].

It is public knowledge that there is huge demand for the production of high-quality food. Many of the tests available for testing, for example, whether a food of bovine origin has been adulterated, include DNA, protein and fat analysis. However, there are still limitations in terms of reproducibility and repeatability. That is why techniques based on DNA analysis have become increasingly relevant and the qPCR appears among the mostly named techniques [26].

Biometrology in pharmaceutical biotechnology

Pharmaceutical biotechnology can be defined as the set of all the technologies needed to produce biopharmaceutical products. Thanks to the advances in biotechnology, we have practically strayed from animal proteins to produce proteins *"custom made"* with the human amino acid sequence itself. Over the past 30 years, biopharmaceutical production has been rising and today we have a wide variety of medicines including proteins, RNA, hormones, antibodies, DNA sequence for gene therapy, among others. All these products are produced on an industrial scale and biometrology occupies a role in quality control. As we already mentioned, everything must be measured, validated and have the exact chemical composition to produce a benefit in humans making sure to preserve the quality of their life [27].

Biometrology in the environment

Monitoring of exposure to GMOs in the air as a result of leakage in containment in laboratory equipment or bioreactors should be fast, specific, sensitive and simple. In 1997, H. Parkes took part in a project that analyzed two equipment commonly used for bioaerosol sampling. They developed PCR-based detection methods for discrimination and quantification of genetically modified and non-modified *E. coli* cells captured by both pieces of equipment [28].

Biometrology in biosciences

The application of biometrological concepts across all lines of research involves the use of validated primary reference methods and certified reference materials (CRM). The latter refers to materials where their composition and properties are very well characterized**,** which ensures greater confidence and comparability of the results. Over the past few decades, NMI has developed a huge variety of CRM, many of which are available on the market.

In the field of biochemistry, the quantification of certain biomarkers allows the detection of most common diseases in humans. Much of the reliability of the results is due to the use of reference methods that are validated internationally. All of them can be found in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database and a list of primary reference methods is displayed depending on the type of biological entity you want to quantify [29].

Biometrology: A New Challenge

When we talk about metrology and its application areas, it is perfectly natural for biotechnologists to think that there is no relationship with their work. Many are unaware of the activities of this branch of science, and when they begin to understand it, they almost immediately tend to associate this is an issue that belongs to physicist and engineers. They think the measurements are none of their business, however metrology is present in the laboratory all the time. Awareness of the measurements we make daily, focusing on the context where they are being carried out, the condition of the equipment, the protocols we employ, lead us to obtain increasingly accurate and precise results. Saying something is accurate denotes reliable results, but what determines that degree of confidence? One of the main responsible factors is the uncertainty of the measurement, that is, the degree of certainty we have around the measured value. With this definition in mind, one can ask what causes the variability of results. The answer can be very broad, because the uncertainty value is composed of all those sources of error that are part of the measurement process. All of them will finally be reflected in the uncertainty value and, therefore, in the degree of confidence of our results. Now, one can imagine that getting accurate and precise results, everything will be solved, but what certifies that those results are correct? The answer to this question is the reproducibility and repeatability of the measurement. Many times, in the field of biosciences these last two concepts are not possible and that is where biometrology comes to its place. Irreproducibility commonly occurs when trying to reproduce experiments that were previously conducted by other scientists or by repeating experiments using reagents from different suppliers. We often cannot understand the reasons for the differences observed and, in the need to publish or secure external funding, we continue to work and conclude with data whose veracity is not reliable. Another very important reason is the complexity of biological systems involving a myriad of interactions that are often susceptible to any variation in the environment. So, it is very common to think that it is impossible to apply metrological concepts to biological systems because of the huge number of variants they present. However, it is far from being impossible and today many research groups are dedicating all their efforts to achieve global standardization that results in producing equivalent results [30,31]. Other major challenges of biometrology are measurements in dynamic systems, where metabolic pathways are independent, subtle processes of molecular recognition and interaction are occurring, and denaturation and post-traductional modifications of proteins are possible [32].

So far, we have seen that biometrology is a nascent science, there is still a lot of work ahead. Fortunately, more and more scientists are aware of the importance of measurement processes, of knowing the properties and composition of the measuring very well, of the need for certified reference materials that allow to achieve reproducible results in any part of the world. So, what is biometrology? On April 12th, 2017, at a Massive Analysis and Quality Control Society Conference in the United States, it was presented a definition establishing that Biometrology is the science of biomeasurement to enable the global comparability of bioanalytical measurement results of biological characteristics value, with traceability to international SI, legal unit or internationally agreed unit. Finally, what can be quantified? Right now, the answer goes all the way to the limits that existing technology imposes. However, the list is endless, and this is the challenge ahead of biometrology.

Conclusions

A revolution was necessary for two units: the meter and the kilogram to be universally defined and finally the conflicts in commercial transactions ceased at that moment. A historical journey to what we know today as the SI, has allowed us to understand the relevance of the existence of international organizations that have the responsibility of maintaining and carrying out the units.

Metrology is the science of measurements and is not a matter that concerns only physicists. Its field of application is very diverse, and biotechnology is among them. Thinking about the link between these two sciences may be unknown, however, it has been developing since the mid-90s. Biometrologists have been conducting metrological research focused on the biosciences. They are currently divided into three working groups: nucleic acid analysis, protein analysis and cell analysis.

Helen Parkes was one of the pioneers in leading research projects that focused on the development of a biometrological infrastructure. Always in search of the comparability of measurements, her main works focused on the quantification of DNA, proteins and lipids and the definition of standard measurement methods.

Finally, biometrology is the science of biomeasurements and in recent years, biometrologists has been working on the validation of measurements for the quantification of entities of biological origin. However, much remains to be done and much of it is due to the lack of knowledge of this science. This article was intended to bring the readers closer to a world they may not know and show them that metrology and biotechnology are two sciences that work together, that it is a reality and that is far from utopian.

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