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FEATURE ARTICLE

Reliable biological and multi-omics research through biometrology

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Abstract

Metrology is the science of measurement and its applications, whereas biometrology is the science of biological measurement and its applications. Biometrology aims to achieve accuracy and consistency of biological measurements by focusing on the development of metrological traceability, biological reference measurement procedures, and reference materials. Irreproducibility of biological and multi-omics research results from different laboratories, platforms, and analysis methods is hampering the translation of research into clinical uses and can often be attributed to the lack of biologists' attention to the general principles of metrology. In this paper, the progresses of biometrology including metrology on nucleic acid, protein, and cell measurements and its impacts on the improvement of reliability and comparability in biological research are reviewed. Challenges in obtaining more reliable biological and multi-omics measurements due to the lack of primary reference measurement procedures and new standards for biological reference materials faced by biometrology are discussed. In the future, in addition to establishing reliable reference measurement procedures, developing reference materials from single or multiple parameters to multi-omics scale should be emphasized. Thinking in way of biometrology is warranted for facilitating the translation of high-throughput omics research into clinical practices.

Keywords Biometrology · Reference materials · Measurement uncertainty · Metrological traceability · Multi-omics · Reproducibility

Introduction

Metrology is the scientific study of measurement and its application. By clearly defining the units and, thus, providing a common basis [1, 2], it enables reliable and comparable results independent of where, when, and by whom the measurements are performed. Therefore, it is important that science and technology prioritize metrological developments if their impact is to be maximized. Metrology shapes not only scientific activities, but also anything that relies on agreed-upon measurement systems across the world including trade, manufacturing, air travel, satellite communication,

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agriculture, and medicine as well as management of the climate and environment [3, 4]. The importance of standardized units has been recognized for a long time, as early as the standardization of Chinese weights and measures by Qin Shi Huang in 221 BC. During the late eighteenth century, countries around the world began to adopt standard units of length and weight after the French Revolution [5]. By the end of the nineteenth century, 17 states signed the Treaty of the Metre in Paris and established the International Bureau of Weights and Measures (BIPM) [6, 7]. The original purpose of BIPM is to maintain standards of meter and kilogram and to enable the international implementation of these standards. With the development of global societies, the number of member states of BIPM has grown to 64, and the International System of Units (SI) has been developed and defined. This system is based on the seven base units, i.e., second, ampere, mole, kelvin, and candela, in addition to meter and kilogram [8, 9].

Metrology goes far beyond the definition of units; scientists are concerned with how to make the measurements more reliable and comparable over space and time. The metrological mindset is attention to details and relationships between the measurements being made to estimate a given value and the true value. This underpins the confidence needed for obtaining accurate results to support robust conclusions in any field of science and becomes more relevant as increasingly precise measurements are demanded as a given field develops such as in biology today [3, 10]. Although many biologists consider technical variations and analytical sensitivities when biological measurements are performed, few systematic studies have been conducted to identify and quantify the factors influencing the accuracy of the measurements they make. Identifying the contributions to the "measurement uncertainty" is one of the core concerns of metrology [11, 12].

Measurement uncertainty is the statistical expression of the dispersion of results when measuring a given quantity. For measurements in physics, metrology has provided best practices for uncertainty analysis [13, 14]. However, for biological measurements, there are numerous uncontrolled and unknown variables that can affect the measurement results, which renders the estimation of measurement uncertainty for biological experiments difficult [15]. This is exacerbated by the fact that metrology and measurement uncertainty are not commonly used to describe or understand the accuracy of a given measurement in biology: the use of metrology in the life sciences is in its infancy. Nevertheless, the principles of uncertainty analysis can provide biologists the guidance for assessing how reliable a measurement is and where improvements are required. Due to biological variability and complexity, one of the core concerns of biometrology is the comparability of measurements and measurement results, the lack of which could lead to inaccurate measurements and ultimately wrong findings. This is becoming increasingly important as life scientists, industries, and clinicians push the boundaries of biological research using evermore precise and high-throughput analytical tools to get more real-world data [16, 17].

Improved accuracy in biological measurement can best be achieved by establishing traceability to a common reference, e.g., the SI [18]. Metrological traceability is a property of a measurement result whereby the result can be related to a stated reference through a documented unbroken chain of calibration, each contributing to the measurement uncertainty [19]. Metrological traceability ensures that different measurements can be compared; e.g., USB ports made by countries in Asia have the same dimensions as those made in Europe or the Americas [20]. In chemistry, traceability is usually achieved through a series of reference materials supported by measurement procedures starting with the analyte in pure form certified using a primary reference measurement procedure, which in turn is then used to certify the analyte in relevant matrix materials that can be used in routine measurements [21, 22], such as glucose in serum and pesticides in food. However, one of the main challenges in biological measurements is already the unambiguous definition of the measurand. Even for relatively simple measurements, such as the level of a specific surface marker on a particular immune cell, the fundamental truth is difficult to define, which is the key problem that needs to be solved urgently by biometrology. This review summarizes the current research progress and challenges as well as future perspective of biometrology to promote an awareness for the valuable contribution metrological concepts can provide for biology. It puts forward countermeasures and suggestions in order to provide reference for the development of biometrology.

Research advances in biometrology

History of biometrology

Biometrology, an emerging field in metrology, is a discipline providing comparability and reliability to biological measurement and its applications by ensuring traceability to a common standard. It mainly focuses on the development of biological reference measurement procedures/methods and reference materials to establish traceability of the measurement results. The purpose is to achieve accuracy and consistency of biological measurements across the world and to ensure that the measurement results can be traced to the SI units or internationally recognized units. In October 1999, during the XXI General Conference for Weights and Measures (CGPM), an ad hoc biometrology task group was established to support biological

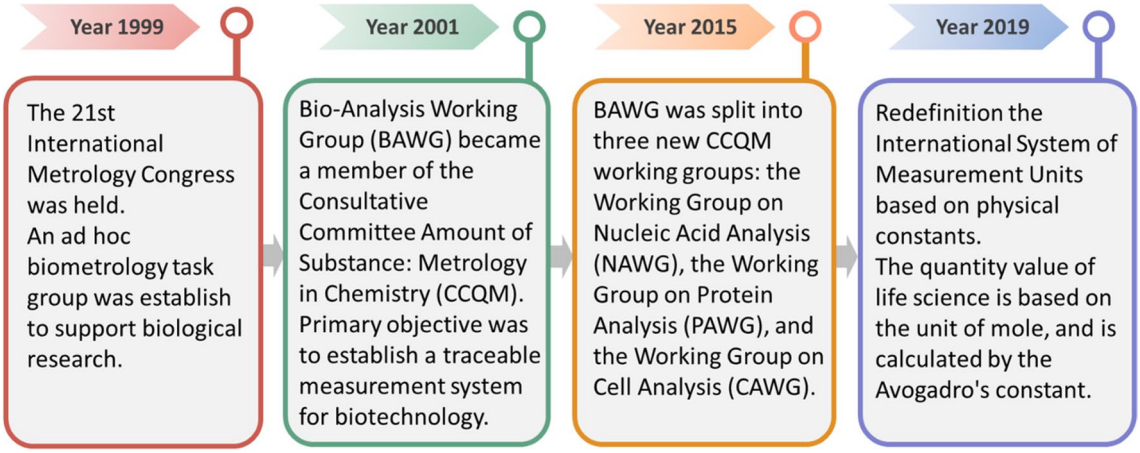

Fig. 1 History of the development of international biometrology

research (Fig. 1). In 2001, it led to the founding of the Bio-Analysis Working Group (BAWG) that became a member of the Consultative Committee for Amount of Substance: Metrology in Chemistry (CCQM). The primary focus of BAWG was to establish a traceability chain for measurements of quantities in biological systems including biotechnology, medicine, and food testing. The priorities were on measurements of nucleic acids and proteins and the standardization of measurement methods and analytical processes, as well as on the support on the development of reference materials. Based on the needs of genetically modified organisms (GMO) labeling requirements, BAWG firstly proposed a series of pilot (P) and key (K) comparisons of measurements of GMO, including direct quantification of DNA [23] (CCQM P44, CCQM P60, and CCQM K61) and quantification of genomic DNA fragments extracted from a biological tissue [24] (CCQM P113 and CCQM K86). These comparisons demonstrated the comparability and consistency of National Measurement Institutes/Designated Institutes (NMIs/DIs) in GMO measurements, thus avoiding international trade disputes. Aiming at the consistency of measurements of DNA methylation, BAWG started a series of comparisons [25] (CCQM P94, CCQM P94.1, and CCQM P94.2). For RNA measurements, pilot comparisons of in vitro-transcribed RNA [26] (CCQM P103 and CCQM P103.1) were conducted. With the rapid development of biotechnology and the need for biometrology, the Consultative Committee for Amount of Substance: Metrology in Chemistry was renamed as Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) in October 2015, and the BAWG was split into three new CCQM working groups according to their major analytes: the Working Group on Nucleic Acid Analysis (NAWG), the Working Group on Protein Analysis (PAWG), and the Working Group on Cell Analysis (CAWG).

Reliable nucleic acid analysis through metrology

Nucleic acid metrology is a branch of biometrology that studies unity of measurement units and accurate and reliable values of nucleic acid measurement, including quantitative measurements and qualitative examination of the property values (the measured values and nominal properties) of nucleoside sequences and modifications. The priority of nucleic acid metrology is to establish the traceability and reference measurement methods for property values of nucleic acids, and the purpose is to achieve accuracy and consistency of measurements internationally and to ensure that the measurement results can be traced to SI or internationally recognized units. Therefore, the focus of NAWG is to carry out key comparisons and pilot comparisons related to nucleic acid measurement and to evaluate and benchmark the competences of NMIs/DIs for nucleic acid analysis [27, 28]. This includes analysis of DNA and RNA and their modifications as shown in Fig. 2a.

At present, focusing on quantification of nucleic acids, NMIs/DIs have successively developed the ability to make high-accuracy measurements of nucleic acids using techniques including high-performance liquid chromatography–isotope dilution mass spectrometry (HPLC-IDMS) [29, 30], inductively coupled plasma–atomic emission spectroscopy (ICP-AES) [31], inductively coupled plasma–mass spectrometry (ICP-MS) [32], and single-molecule counting [33, 34] when targeting high-concentration pure materials.

The NAWG has also been instrumental in recognizing the potential of digital PCR (dPCR) to perform with very high accuracy [29, 35, 36], which differs from the above methods as it is able to perform with high accuracy on dilute extracts in complex mixtures of non-target nucleic acids. dPCR has the potential to transform applied nucleic acid analysis as it can make high-accuracy measurements on real specimens lower down the traceability chain.

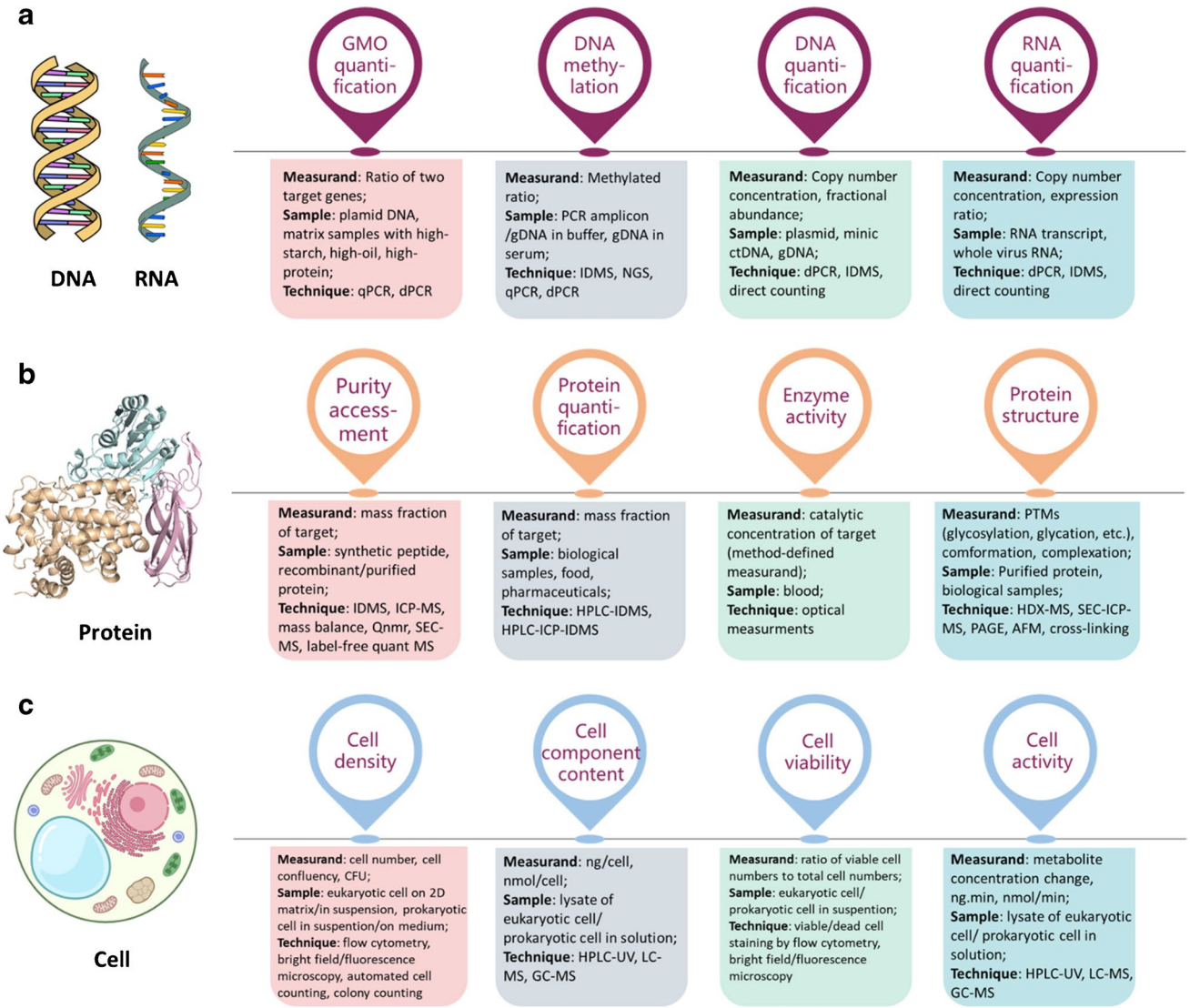

Fig. 2 Research advances including the defined measurand, analysis of sample types, and techniques used for the quantitative and qualitative measurements in **a** nucleic acid metrology, **b** protein metrology, and **c** research advances in cell metrology

BAWG/NAWG organized a total of 20 international comparisons [24, 26, 28, 35, 37, 38] of oligonucleotides, plasmid DNA, DNA methylation, RNA biomarkers, pathogenic nucleic acids, and GMO as summarized in Table 1; accurate quantification of linear plasmid DNA and RNA and accurate quantification of the relative transgenic contents in high-starch and high-fat transgenic plants were ensured in consequence. During the COVID-19 pandemic, the international comparison on measurements of the copy number concentration of the SARS-CoV-2 (CCQM-P199.b) was rapidly conducted by NAWG and coordinated by the National Measurement Laboratory (LGC, UK), the National Institute of Metrology (NIM, China), the National Institute for Biological Standards and Control (NIBSC, UK), and the National Institute of Standards and Technology (NIST, USA). The measurement results were within ± 40% of mean with global equivalence [39]. To further investigate whether reverse transcription efficiency affects the measurements and support a calibration measurement capability (CMC) claim, a new key comparison (CCQM-K181) is currently in progress.

At the same time, to solve the bottleneck problem of genetic testing in clinical applications, NAWG also organized comparisons on measurements of targeted genes in precision medicine [28]. With the increasing use of circulating tumor DNA (ctDNA) sequencing in the clinical management of cancer patients, the accuracy and reliability of ctDNA detection have caused widespread concern [40]. The Sequencing Quality Control Project Phase 2 (SEQC2) Oncopanel Sequencing Working Group [41] evaluated the analytical performance of five industry-leading ctDNA assays

Table 1 International comparability studies on the quantification of nucleic acids

Measurand	Time	Study ID	Coordinator	Description of study
DNA copy number concentration in buffer solution (copy/μL)	2003	CCQM-P44	LGC, NIST	Study on comparability of purified plasmid DNA quantification by qPCR for GMO measurement
	2004	CCQM-P44.1	LGC, NIST	
	2007	CCQM-P60	IRMM	
	2007	CCQM-K61/P44.2	LGC, NIST	
DNA copy number ratio of two targets in matrix samples (%)	2008	CCQM-P113	JRC, IRMM	Relative quantification of TC1507 in GM maize matrix sample
	2010	CCQM-K86/P113.1	JRC, IRMM	Relative quantification of Mon810 in GM maize matrix sample
	2014	CCQM-K86.b/P113.2	NIM, GLHK	Relative quantification of Bt63 in GM rice matrix sample
	2016	CCQM-K86.c/P113.3	JRC, IRMM, NRC	Relative quantification of genomic DNA fragments extracted from a high oil matrix
	2020	CCQM-K86.d/P113.4	UME, NIMT	DNA ratio in a high protein matrix: relative quantification and fractional abundance of genomic DNA extracted from a biological tissue
20 bp oligo DNA concentration (ng/μL)	2006	CCQM-P54.1	LGC	Study on comparability of oligo DNA quantification in buffer solution
Linearized and supercoil plasmid DNA copy number concentration (copy/μL)	2013	CCQM-P154	KRISS	Absolute quantification of DNA in buffer solution
DNA methylation ratio (%)	2007	CCQM-P94	KRISS	Quantification of DNA methylation in a PCR amplicon in buffer solution
	2013	CCQM-P94.2	KRISS	Quantification of DNA methylation in a PCR amplicon in buffer solution
	2022	CCQM-P94.3	KRISS, UME	Quantitative analysis of DNA methylation of a defined human genomic DNA region in buffer solution
Cancer biomarker RNA copy number concentration in buffer solution (copy/μL)	2009	CCQM-P103	LGC	Measurement of multiplexed biomarker panel of RNA transcripts in buffer solution
	2012	CCQM-103.1	LGC	Measurement of multiplexed biomarker panel of RNA transcripts in buffer solution
	2015	CCQM- P155	LGC	Multiple cancer cell biomarker measurement in buffer solution
Wild type and mutant copy number concentration of cancer biomarker (copy/μL) and mutant fractional abundance (%)	2017	CCQM- P184	NMIA, LGC	BRAF V600E and EGFR 19DEL Copy number concentration and fractional abundance of a mutation (SNV or INDEL) mixed with WT DNA in buffer solution
Cancer biomarker copy number concentration and ratio of two target genes (no unit)	2020	CCQM-K176/P218	NIM, LGC	Breast cancer biomarker *HER2* copy number variation (CNV) measurement in buffer solution
Pathogen RNA copy number in buffer solution (copy/μL)	2019	CCQM-P199	LGC, NIBSC	HIV-1 RNA copy number quantification in buffer solution
	2020	CCQM-P199.b	LGC, NIM, NIBSC, NIST	SARS-CoV-2 RNA copy number quantification in buffer solution
	2022	CCQM-K181	NIM, LGC, NIBSC, NIST	SARS-CoV-2 RNA copy number quantification in buffer solution

LGC Laboratory of the Government Chemist; *NIST* National Institute of Standards and Technology; *KRISS* Korea Research Institute of Standards and Science; *IRMM* Institute of Reference Materials and Measurement; *JRC* European Commission's Joint Research Centre; *GLHK* Hong Kong Government Laboratory; *NRC* National Research Council Canada; *NMIA* National Measurement Institute, Australia; *NIBSC* National Institute for Biological Standards and Control; *UME* National Metrology Institute of Turkey; *NIMT* National Institute of Metrology (Thailand); *NIM* National Institute of Metrology (China)

with simulations, synthetic DNA spike-in experiments, and proficiency testing on standardized cell line-derived reference samples. The results showed that above 0.5% variant allele frequency, ctDNA mutations were detected with high sensitivity, precision, and reproducibility by all five assays. Below this limit, detection became unreliable and varied widely between assays, especially when input material was limited. In addition, the false-negatives were more common than the false-positives, which indicated that the accurate detection of low-level ctDNA is the most critical challenge for ctDNA detection.

On the other hand, comparison of measurements of nucleotide sequence has not yet been carried out by NAWG, but in the face of the urgent need, the Genome in a Bottle (GIAB) consortium hosted by NIST was established. The main task of GIAB is to develop reference materials and reference data of the whole human genome, and the first reference material (RM8398) was issued in 2014 [42]. With the development of sequencing technologies, the high-confidence reference data of RM8398 is constantly updated, which now covers 83.8% of the human genome (Grch38) [43]. In addition, GIAB has also developed reference datasets of Ashkenazi human [44] and Han Chinese trio [45], as well as the best practice for analyzing germline variation [46]. The Australian research team developed internal reference standards for DNA [47] and RNA [48] sequencing. NIM and Fudan University have developed the Chinese Quartet multi-omics reference materials (identical twin family) shown in Fig. 3, including reference materials and standard reference datasets for genome [49], transcriptome [50], proteome [51], and metabolome [52]. Besides high-quality haplotype-resolved assemblies, a variant benchmark for two Chinese monozygotic twin samples was provided, which could be used to evaluate the performance of analysis pipelines and sequencing technologies [53]. It is believed that with the extensive application of these reference materials, the reliability of nucleotide sequence analysis will be improved significantly, which will contribute to the rapid development and clinical application of gene sequencing.

Improved protein purity assessment and quantitation through metrology

The goal of protein metrology is to achieve accurate and internationally consistent measurements of protein property values and ensure that the measurement results can be traced to the SI or internationally recognized units. The most important properties in biological research, clinical diagnostics, and biopharmaceuticals are protein content, molecular weight, activity, function, sequence, and advanced structure. To establish traceability for protein measurement results, PAWG focuses on (1) the development and validation of reference measurement procedures for the purity assessment and quantification of high purity peptides and protein CRMs and (2) qualitative and quantitative measurements of peptides and proteins in complex biological matrices and biopharmaceuticals (Fig. 2b). PAWG strives to enable the NMI/DI of its member states to provide the full range of services required to meet industrial and clinical needs [54]. This requires also a close cooperation with other international organizations concerned with the standardization and/or harmonization of clinical markers such as the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the Joint Committee for Traceability in Laboratory Medicine (JCTLM), the International Council for Standardization in Haematology (ICSH), and the International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR).

Similar to the approach used in chemistry, PAWG aims to establish the traceability of results for measurements in biological matrices to the SI via pure peptide and protein materials as primary calibrators [55]. This requires the unambiguous definition of the measurand as well as the production and characterization of the pure material. At present, methods used for the protein characterization and purity assessment are IDMS [56], mass balance [57], quantitative nuclear magnetic resonance (qNMR) [58], and size exclusion chromatography (SEC) either with UV–Vis or MS detection [59]. A potential upcoming method for quantification of amino acid enantiomers and amino acid purity might be high-performance liquid chromatography–circular dichroism (HPLC-CD) [60]. So far, this could be achieved and proven in key comparisons organized by BAWG/PAWG for synthetic human C-peptide [61] (CCQM K115/P55.2), synthetic oxytocin [62] (CCQM K115.b/P55.2.b), and the non-glycated (CCQM K115.2018/P55.2.2018) as well as the glycated hexapeptide of HbA1c [63] (CCQM K115.c/P55.2.c) (Table 2). A first step to characterize larger proteins was the study on the determination of the mass fraction of purity-assessed recombinant insulin in an aqueous calibration solution (CCQM K151/P191) [64]. In response to the COVID-19 pandemic, the NIM, BIPM, and National Research Council of Canada (NRC) jointly coordinated an international comparison for the assessment of the purity of monoclonal antibodies of SARS-CoV-2 (CCQM-P216) [65], which showed that agreement between nearly all laboratories for the amino acid analysis within 2% to 2.5% was achieved. This study has improved national capabilities in the measurement of monoclonal antibodies and has provided technical support to improve the accuracy of antibody measurements for SARS-CoV-2.

Even more challenging is the reliable and comparable quantification of natural peptides/proteins in biological samples such as serum, blood, or urine. Pilot studies have been organized by PAWG for human growth hormone in serum (CCQM P164, recently followed by the key

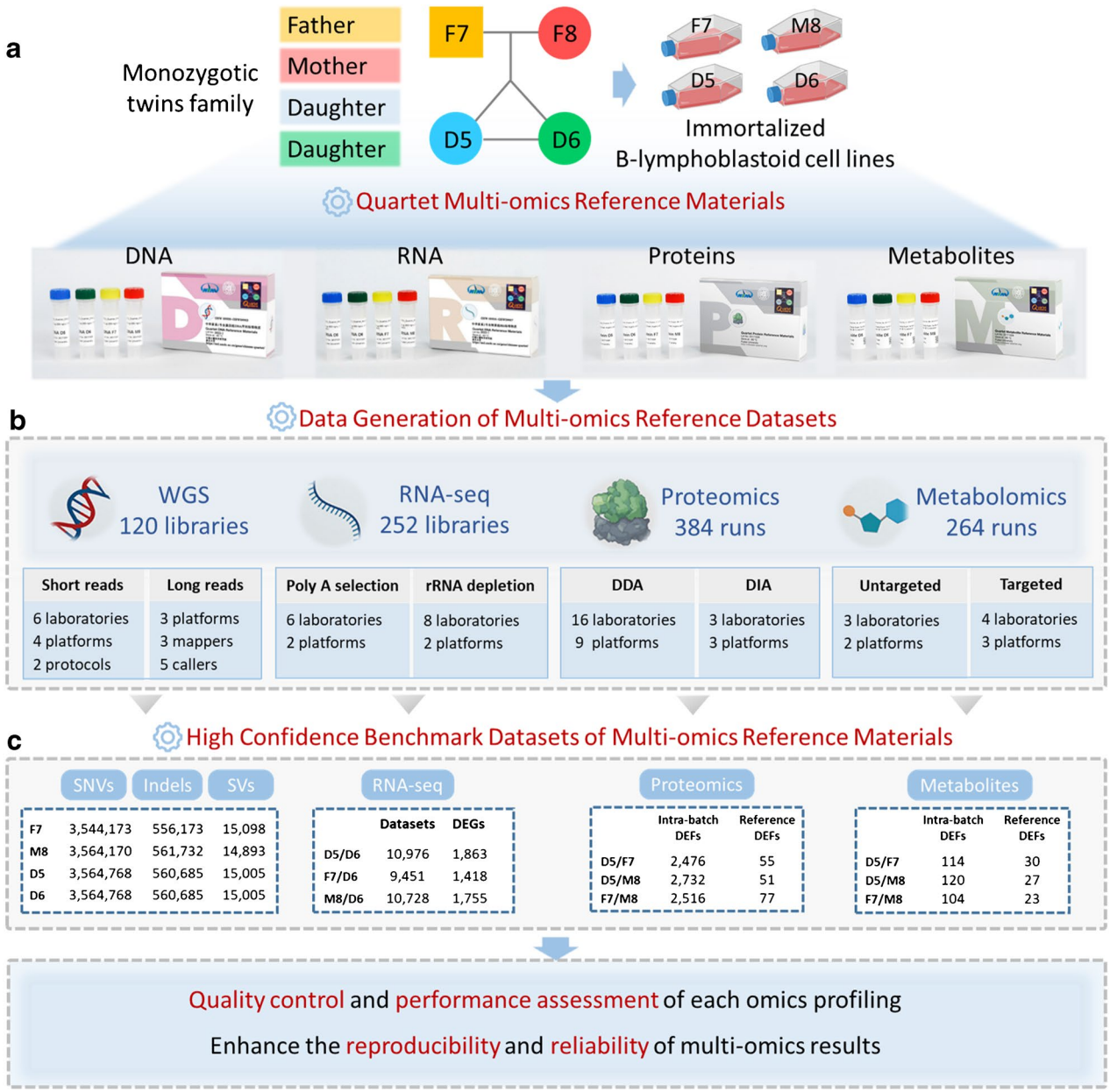

Fig. 3 Overview of the Chinese Quartet multi-omics reference materials and benchmark datasets. **a** Design of Chinese Quartet multi-omics reference materials. Multi-omics refers to genome, transcriptome, proteome, and metabolome. **b** Design and data generation of Chinese Quartet multi-omics reference datasets. WGS, whole-genome sequencing; RNA-seq, RNA sequencing; DDA, data-dependent acquisition; DIA, data-independent acquisition. The WGS, RNA-seq, proteomics, and metabolomics data were generated across different laboratories, platforms, and protocols. **c** High-confidence benchmark datasets of Chinese Quartet multi-omics reference material. SNV, small variants; indels, insertion and deletion with size less than 50 bp; SVs, structural variants with size over 50 bp; DEGs, differentially expressed genes; DEFs, differentially expressed features. The data resources accessed from the Quartet Project Data Portal (http://www.chinese-quartet.org/)

comparison CCQM K177) and total hemoglobin (CCQM P201) as well as the ratio of HbA1c/(HbA1c + HbA0) (CCQM P219) in blood. Methods applied are IDMS and ICP-IDMS, with the latter especially suited for the detection and quantification of metal containing proteins [66]. Various reference materials for pure proteins or proteins in matrix have been developed and are listed in the JCTLM database [67].

Up to now, the method used to ensure traceability is IDMS either via isotopically labeled specific peptides or isotopically enriched metals in metalloproteins. However, this allows only to distinguish the proteins according to their amino acid

Table 2 International comparability studies on the quantification of proteins

Measurand	Time	Study ID	Coordinator	Description of study
Mass fraction of target peptide and impurities in synthetic peptide	2014	CCQM-K115	BIPM	Peptide purity, synthetic human C-peptide
	2018	CCQM-K115.2018	BIPM, NIM, HSA	Peptide purity determination, hexapeptide of HbA0 (VHLTPE or VE)
	2018	CCQM-K115.b	BIPM, NIM	Peptide purity determination, synthetic oxytocin (OXT)
	2018	CCQM-K115.c	BIPM, NIM, HSA	Peptide purity determination-glycated hexapeptide of HbA1c (glcVHLTPE or GE)
	2018	CCQM-P55.2.b	BIPM, NIM	Peptide purity, synthetic oxytocin
	2019	CCQM-P55.2.c	BIPM, NIM, HSA	Peptide purity-glycated hexapeptide of HbA1c
	2019	CCQM-P55.2.2018	BIPM, NIM, HSA	Peptide purity, hexapeptide of HbA0
Protein mass fraction in buffer solution	2017	CCQM-K151	KRISS	Protein quantification: purity-assessed recombinant protein contents in buffer solution using insulin analog
Protein mass fraction in serum	2023	CCQM-K177	PTB	Human growth hormone in serum
Mass fraction of peptide in solution using amino acid analysis	2004	CCQM-P55	LGC	Peptide/ protein quantification
	2008	CCQM-P55.1	LGC	Peptide/protein quantification (repeat)
Mass fraction of protein in whole blood	2019	CCQM-P201	PTB	Quantification of total hemoglobin in blood
Mass fraction of spiked recombinant protein in serum	2020	CCQM-P164	PTB	Growth hormone in serum
Mass fraction of amino acids and peptides specific for constant region released from mAb in buffer solution	2020	CCQM-P216-Part 1	NIM, NRC, BIPM	Quantification of SARS-CoV-2 monoclonal antibody
Ratio of HbA1c/(HbA1c + HbA0) in human hemolysate	2021	CCQM-P219	HSA, LNE, NIM, KRISS	Determination of amount-of-substance fraction of hemoglobin A1c (HbA1c) in human hemolysate
Mass concentration of mAb in buffer solution (UV–Vis), size heterogeneity, mass fraction of peptides specific for variable region and of monomeric mAb	2022	CCQM-P216-Part 2	NIM, NRC, BIPM	Quantification of SARS-CoV-2 monoclonal antibody

BIPM Bureau International des Poids et Mesures; *HSA* Health Sciences Authority; *LNE* Laboratoire national de métrologie et d'essais; *PTB* Physikalisch-Technische Bundesanstalt

sequence or the incorporated metal. As a protein usually exists in a multitude of different variations, the sum of all forms that contain these peptides or the metal in common might not be the biological relevant parameter as the different forms can have different biological functions. At present, measurement is still based on the primary structure of proteins (the amino acid sequence), but protein activity plays more important role in practical applications such as biomedicine and in vitro diagnostics (IVD), which is closely related to the three-dimensional structure of proteins. Future challenges will be to detect and possibly quantify structural properties including epitope mapping, molecular assemblies such as lipoprotein particles, and posttranslational modifications such as glycosylation or phosphorylation. Methods that can be used are ion mobility spectrometry-MS (IMS-MS) [68], hydrogen–deuterium exchange MS (HDX-MS) [69], electrospray–differential mobility analysis–condensation particle counter (ES-DMA-CPC) [70, 71], and surface-enhanced Raman spectroscopy (SERS) [72, 73]. Currently, the measurements of enzyme activities are method-defined measurands [74, 75]. If it would be possible to determine the activity of proteins based on establishing the structure–activity relationship, this might allow establishing SI traceability for these measurement results [76], which is still being explored in research. For achieving standardization, there is still a long way to go. Furthermore, as the number of proteins used as clinical markers or in biotechnology is rapidly increasing, calibration-free and rapid methods might become more important in the future, and traceability has to be established for the results obtained with these methods [77]. Other challenges are the often very low concentrations and the dynamic of proteins in biological samples as well as the fingerprinting of the proteome. Methods such as single-molecule counting and antibody-based nucleic acid Olink detection [78] techniques might be the answer. In addition, the single-molecule nanopore-based protein sequencing technology developed by researchers of Delft University of Technology has attracted a lot of attention [79]. The technology can successfully scan and read the amino acid sequence of a single protein through the narrow structure of the nanopore and monitor the ion current to resolve the differences in amino acid sequence along the peptide main chain. This may have a significant impact on protein analysis and medical diagnosis and, thus, bring new ideas for protein measurement. It will also be a challenge to provide reference materials for these emerging fields which are well characterized and stable to be used at the different levels of the traceability chain.

Better cell counting and activity measurement through metrology

Cell metrology is a branch of biometrology and an academic discipline about the measurement of cells (including eukaryotic cells and prokaryotic cells) and its applications. Cell metrology studies the unification of measurement units and ensures the accuracy and reliability of the measurement results in the field of cell measurement. Cell metrology research includes cell measurement theory, standards, and techniques, in order to realize the accuracy and consistency of measured values and nominal properties of cells and to ensure that the measurement results can be traced to the SI, the internationally recognized unit or the agreed measurement procedure/protocol. Divided by the measurands, cell metrology includes the quantitative measurements of intact cell density/concentration, cell component content, cell viability, and biological activity, summarized in Fig. 2c, as well as the qualitative measurements of cell morphology, genetics, physiological function (such as stem cell differentiation potential), and other biological characteristics.

For blood cell measurements, which is widely applied in clinical diagnosis, basic metrological research based on flow cytometry and microscopic measurement is carried out. Reference measurement procedures are developed to realize accurate counting and typing of clinical cells [80, 81]. A flow cytometry measurement method for white blood cell typing based on microscopic imaging flow cytometry was established [82], and reference materials for absolute counting of CD4 + positive cells, urine sediment cell typing [83], and live/dead cell counting were developed. The booming cell therapy industry has put forward an urgent metrological demand for the characterization of high-quality cell therapy products. For the variability of cell number and cell viability in cell therapy product measurements, inert polymer microspheres and real cell reference materials can provide instrument calibration and method validation [84, 85]. For cell biocompatibility evaluation and drug activity evaluation based on cell activity measurements, a combination measurement system that can quantitatively monitor cell response to external environment and a super resolution microscope that can be used for dynamic imaging of living cells have been developed [86]. A single-cell activity measurement method based on Raman spectroscopy of heavy water metabolism of cells has been established [87]. For the counting of prokaryotic cells such as bacteria, a new method for highly accurate flow measurement of microbial viable bacteria traced back to the natural unit "one" was established [88].

In order to ensure the traceability, accuracy, and comparability of eukaryotic and prokaryotic cell measurements in biomedicine, clinical diagnosis, food safety, biosafety, and other fields, CAWG has organized several international comparisons of eukaryotic and prokaryotic cell measurements (Table 3), such as counting cells with specific phenotypes based on flow cytometry [89] and counting cells with specific phenotypes based on fluorescence microscopy (CCQM P102 counting of CD4 + lymphocytes and CCQM P165 counting of CD34 + hematopoietic stem cells). The current pilot study of CCQM P205 which aims to study the comparability of the filtration method and flow

cytometry to quantify *Escherichia coli* in drinking water and to find a higher order measurement method for microorganism quantification is being organized by NIM.

Challenges of biometrology

Lack of primary reference measurement methods

At present, few reference measurement procedures and methods have been defined as primary methods or potential primary methods in biometrology. For example, IDMS can eliminate factors influencing the analysis process by using isotope-labeled analytes as internal standards, which is considered as the primary method in the field of inorganic chemistry [90, 91]. Similar IDMS methods were established for the measurement of biological macromolecules such as nucleic acids and proteins, but certain factors still cannot be defined clearly. For example, to quantify oligonucleotides and short nucleic acids fragments by IDMS, hydrolysis of nucleic acids by phosphodiesterase is necessary. However, due to the limitation of detection sensitivity of un-hydrolyzed nucleic acids, the measurement of hydrolysis efficiency is not very accurate. In addition, for longer nucleic acid fragment quantification, hydrolysis should be performed after fragmentation, but there is a loss of nucleic acids that cannot be characterized by the existing methods. Moreover, these methods can only quantify pure nucleic acids (nucleic acids without non-target sequences). In view of the complex matrices of organisms or biological samples, the in situ measurement of target nucleic acids still has not been realized. Similar problems exist with protein measurements. When protein quantification is performed by measuring amino acids after hydrolysis, it is necessary to determine whether the protein or polypeptide was hydrolyzed completely and if contaminations in the sample also released amino acids. Determination of digestion efficiency is needed in protein quantification based on specific peptides, which is also limited by the sensitivity of analytical methods.

The only effective way to achieve standardization and comparability for clinical tests is metrological traceability through a reference system including the interconnected reference methods, reference materials, and reference laboratories. Up to date, there are more than 2000 international clinical inspection items, but only 215 reference methods have been released by JCTLM [67]. For clinical nucleic acid tests, there are only two reference methods (KRAS G12D and human cytomegalovirus DNA). Therefore, there is still a long way to go to provide reference measurement procedures for all clinical tests.

Biological reference materials need new norms

With reference to chemical metrology, biometrological researchers firstly developed reference materials in order to establish traceability of biological measurements, such as the measurements of whole-genome sequence [42, 49], protein content [92], and cell phenotype [83, 93]. However, the biological reference materials cannot be reproduced with batch-to-batch consistent characteristics. First of all, biological reference materials are generally from human, cells, or microorganisms, whose characteristics are changing with continuous metabolism of the living organisms. When the source of the reference material changes, it is hard to ensure reproduction of exactly the same material as the previous batches. Moreover, if mutations are introduced during the cell passage, the variation frequency needs to be determined; the nominal properties of cells such as the genome sequence and modification information, mutation sites, and epigenetic information should be measured again, as the new batch cannot be completely consistent with the previous ones. Thus, the duplication and identification of biological reference materials that have obtained certificates need to be distinguished from chemical reference materials, and new batches have to be carefully linked to previous ones.

The uncertainty evaluation of reference materials with quantitative values is usually based on the mathematical model

Table 3 International comparability studies on cell analysis

Measurand	Time	Study ID	Coordinator	Description of study
Specific cell number/mL	2007	CCQM-P102	NIST, NIBSC	Quantification of cells with specific phenotypic characteristics
	2015	CCQM-P165	NIST, PTB, NIBSC	Quantification of CD34 + cells
Cell number/mL	2020	CCQM-P217	NIBSC, NIST	Enumeration of fixed peripheral blood mononuclear cells in suspension
Mimic cell number/mL	2022	CCQM-P222	NMIJ, PTB	Number concentration measurement of particles for cellular analysis
Specific cell number/mm^2	2010	CCQM-P123	INRIM, LGC, NIST	Cell quantification on solid substrate
	2021	CCQM-P197	NPL	Proliferative stem cell number per unit area
Number of *E. coli* in CFU/100 mL	2021	CCQM-P205	NIM, NIST	Quantification of *E. coli* in drinking water

INRIM Istituto Nazionale di Ricerca Metrologica; *NMIJ* National Measurement Institute of Japan

depending on the measurement results. The separately evaluated uncertainties of each parameter are combined to give the uncertainty of the measurement procedure, and the uncertainty introduced by the inhomogeneity and instability of the reference materials should also be considered [12, 94]. However, if the nominal properties of biological reference materials are not specific values, questions arise. How to identify and estimate the uncertainties of this qualitative result? How to combine the uncertainties of inhomogeneity and instability? New approaches must be developed for these properties.

Lack of metrology thinking in multi-omics

Ensuring traceability and defining uncertainty of measurements are the core of metrology thinking. Great progress has been made in traditional single-parameter clinical chemistry test development, where metrological traceability is indispensible [95–97]. The publication of ISO 17511 had an important impact on the development of IVD and laboratory medicine and had greatly promoted the quality improvement of IVD and laboratory medicine [22]. With the development of multi-omics technologies, the laws of life including the occurrence and development of diseases can be explored simultaneously at multi-omics levels including genome, transcriptome, proteome, and metabolome [98–100]. Besides, the 2020 edition of the ISO 17511 standard clarified that it also applied to certain qualitative measurement procedures and emphasized the requirements for metrological traceability of human samples [101], and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) strategy on stimulating the formulation of analytical performance specification fit for the intended use was inspired to build on for multi-omics diagnostics [102]. Indeed, for reliable biological and biomedical measurements, metrological thinking is extremely important and indispensable.

Due to the development of high-throughput experimental technologies, omics data have been promoted from the petabyte (PB)-level based on the genome to the exabyte (EB)-level based on multi-omics [103, 104]. Considering the massive data generation by different analysis methods and algorithms, the reliability of omics results is very important. In practice, all the different technical repetitions, experimental batches, laboratories, platforms, and analysis methods currently lead to irreproducible results due to lack of metrology thinking [41, 105–113]. Irreproducible research leads to a great waste of resources, even endanger the health and lives of patients, which limits the reliable application of omics in research and clinic [15, 114]. How to support the wide use of data analysis and the integration of large data sets by providing traceability to big data and, thus, enable biological and clinical data of high quality are great challenges faced by biometrology.

Medical artificial intelligence and big data are two complementary concepts [115]. The core technology of most medical artificial intelligence is based on big data and machine learning and deep learning using big data, while analysis and processing of the medical big data sometimes rely on medical artificial intelligence [116, 117]. Regardless of medical artificial intelligence or big data, their technical basis is data, which have to be accurate, valid, and FAIR (findable, accessible, interoperable, and reusable) [118], reliable, and reproducible to be of any use. How to serve life sciences by providing the tools to ensure the quality of big data will also be a great challenge for biometrology. Therefore, with the in-depth exploration of the processes of life, there will be more requirements for reference measurement tools and methods, and continuous innovation is always required.

Future trends

Exploring potential primary methods based on interdisciplinary approaches

To meet the challenge of currently insufficient primary methods in biometrology, the advantages of an interdisciplinary approach including physics, chemistry, and biology should be exploited to develop qualitative characterization and quantitative measurement methods for the accurate measurement of the content of biological entities including nucleic acids, proteins, and cells. For example, traceability of the counting method of biological entities such as cells and nucleic acids to the natural unit “one” has been clarified by BIPM [119]. However, there are still unknown factors in the current methods used in counting such as single-molecule counting and dPCR which affects the traceability. Specifically, single-molecule counting is based on binding of fluorescent dyes and DNA or RNA [120, 121], and the binding efficiency can currently only be demonstrated by employing other orthogonal methods. Similarly, dPCR relies on microfluidic technology to distribute nucleic acids into ten thousands of small partitions; whether there is a target molecule in each partition or not should be ensured before PCR amplification and fluorescence detection [122, 123]. From a physical point of view, this is not a counting method at the single-molecule level in real sense, but a corrected counting method based on the Poisson distribution. Biometrology researchers are trying to improve it to serve as a potential primary method or reference measurement method through the reproducible measurements of multiple laboratories and platforms [124–126].

During the COVID-19 pandemic, biological measurements have attracted the attention of researchers engaged in physics and nanomaterials. A quantum sensor was developed based on nitrogen-vacancy centers in diamond, which can measure the SARS-CoV-2 in a short time [127]. Although further studies should be performed to ensure the accuracy and reliability of

this method, the possibility that biomolecules can be detected by clearly described mathematical formulas was demonstrated. As predicted, more new measurement technologies and devices will be developed by interdisciplinary research in the near future. For example, the coherent anti-stokes Raman scattering microscopy was used for imaging of biological macromolecules, which enables qualitative and quantitative measurement based on the spectral characteristics [128, 129]. As recently reported by Martínez-Martín et al. [130], the mass fluctuations of living mammalian cells throughout the cell cycle as well as the influence of culture conditions on the mass of cells can be observed using a picobalance-based ontoptically excited microresonator. Compatible with fluorescence microscopy, fast and subtle mass fluctuations of the living cells were also successfully measured when infected with a virus.

Developing of novel biological reference materials with multiple parameters

Biological reference material is an effective tool for the traceability establishment and reliable measurements of biometrology, but the development is still in its infancy. The characterization of biological substances often requires multi-parametric measurements. For instance, when characterizing a monoclonal antibody drug, it is necessary to determine the physical and chemical properties, quantify the main components and impurity contents, and characterize the antigenic epitopes. Therefore, the traceable value of biological reference materials assigned for a single parameter indeed cannot meet the requirements for quality control for future applications. For the diverse and complex biological substances, it is impossible to develop one-to-one reference materials. However, to control the factors during the entire measurement process, a representative case can be chosen that covers as many biological systems as possible. Thus, novel reference materials with multi-parameters should be developed rather than a single parameter with its uncertainty [131, 132]. For example, the multi-parameters should include physical properties such as purity and quantity; chemical properties such as concentration and structure; and biological properties such as bioactivity (Fig. 4a). Moreover, new breakthroughs are also needed for the evaluation of uncertainty. Using the basic models, extended models, and deep learning models, the combined uncertainty of the novel biological reference materials would be evaluated, also for the reliability of norminal property examination (Fig. 4a). Finally, based on in-depth studies on multi-parametric data analysis methods and reference materials, novel metrological capabilities will be developed to promote the developments in biology, including new computational approaches, technologies, and biology knowledge (Fig. 4b). Besides, with the rapid development of biometrology in many areas of life sciences, in order to elucidate biological truths, it may be necessary to integrate measurement results from different areas. When examining the quality of a cell therapy, the number of receptors per cell

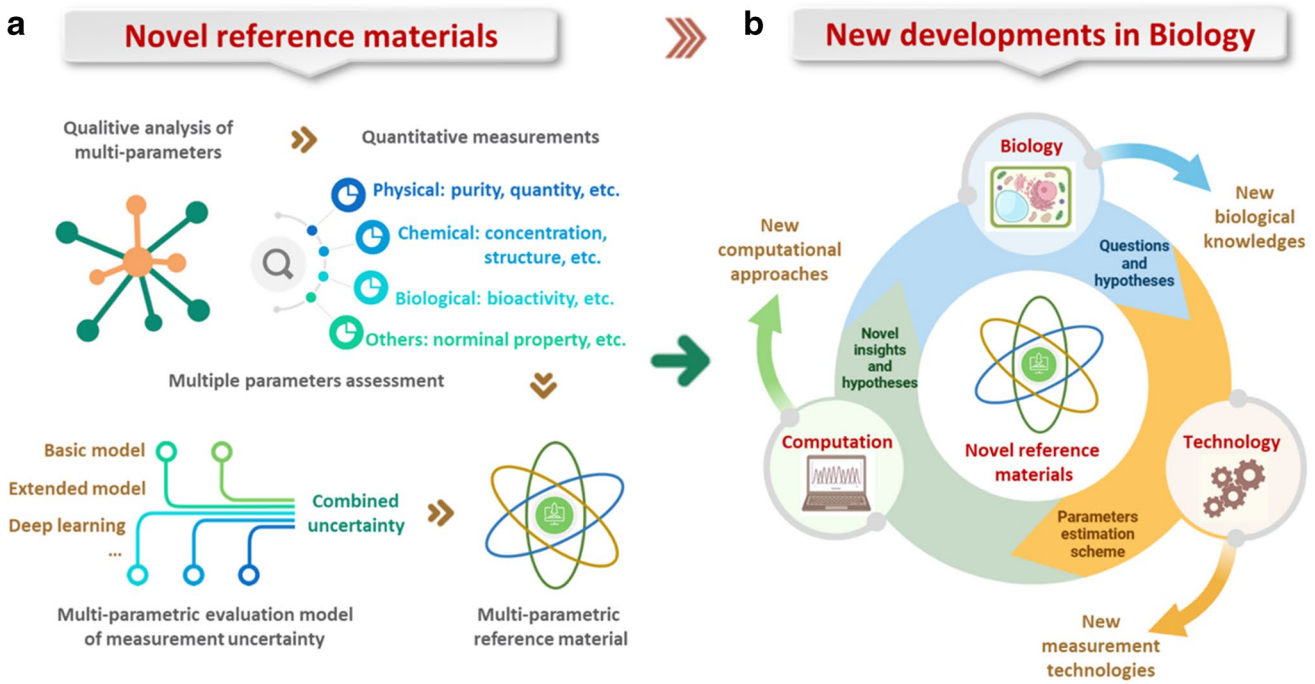

Fig. 4 Development guidance of novel biological reference materials with multiple parameters. **a** Design thinking of novel biological reference materials with multi-dimensional parameter assessment and multi-parametric evaluation model of measurement uncertainty. **b** New developments in biology based on novel metrological thinking

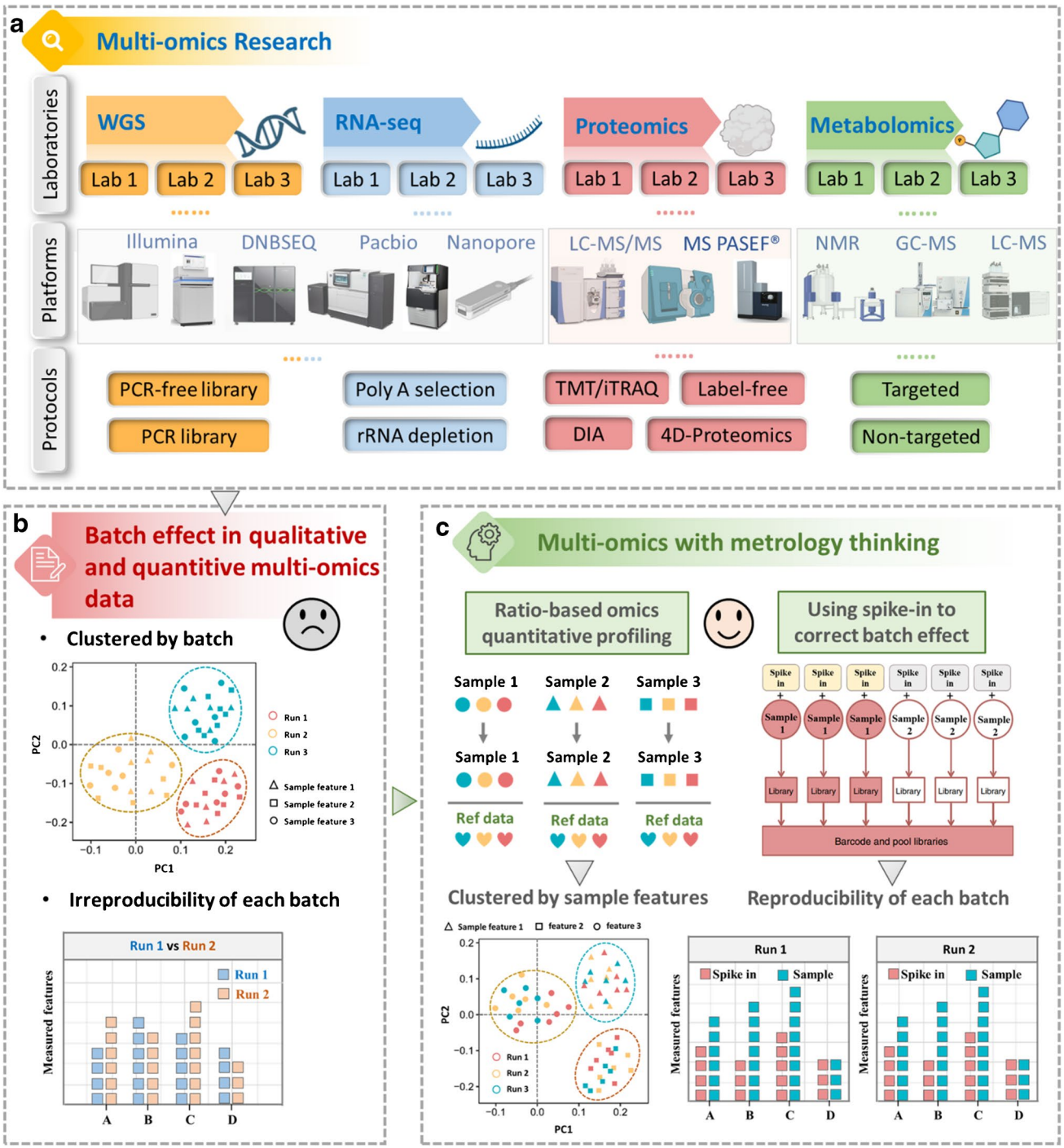

Fig. 5 Reliable and reproducible multi-omics research with metrology thinking. **a** Multi-omics research with different laboratories, platforms, and protocols. WGS, whole-genome sequencing; RNA-seq, RNA sequencing; TMT, tandem mass tag; iTRAQ, isobaric tags for relative and absolute quantitation; DIA, data-independent acquisition. **b** Batch effect in qualitative and quantitative multi-omics data. Data are generated across different laboratories, platforms, and protocols that introduced batch effect; the multi-omics results would be clustered by batch and also caused irreproducibility. **c** Reliable and reproducible omics research with metrology thinking, e.g., using spike-in or ratio-based profiling with universal reference materials for multi-omics and multi-platforms

would be determined by combining of the protein receptor measurement through protein metrology and a cell counting technique through cell metrology. Indeed, only by achieving true multi-dimensional measurement and standardization of measurement procedures can biological reference materials be effectively quality controlled, so as to help clinical diagnosis,

IVD products, etc. Some international comparisons are proposing across the working groups to support multi-parameter measurement.

Metrology thinking supporting reliable and reproducible measurement in multi-omics

Multi-omics has great potential in discovering effective biomarkers for disease treatment and precision medicine. More awareness on the need for metrological traceability is needed all areas of laboratory medicine outside classical single-parameter clinical chemistry, especially in the molecular and multi-omics areas. However, it is very difficult to guarantee the reliability and reproducibility of big data generated by multi-omics techniques without the metrology thinking, let alone the analysis results by diverse pipelines and algorithms [112, 113] (Fig. 5a, b). Therefore, it is extremely important to ensure the reliability and reproducibility of the data production and data analysis in multi-omics research. In order to meet the performance evaluation of multi-omics analysis, multi-omics benchmark including development of multi-omics reference materials and reference measurement procedures is very useful but challenging. Using the current omics technology, it is difficult to accurately measure the absolute gene expression or protein abundance, and to measure thousands of targets by traditional quantitative methods is also not feasible. On the contrary, the ratio of different gene expression or protein abundance is reproducible among different platforms and laboratories (Fig. 5c), no matter which method is used [50, 133]. Therefore, using the ratios between two samples as the reference values of omics reference materials may realize the reliable evaluation of the quantitative omics (transcriptomics, proteomics, and metabolomics) (Fig. 5c), which is significant progress in establishing reference measurement procedures of multi-omics. Additionally, developing a set of well-characterized internal standards to correct batch effect, the true set of multi-omics data would be obtained (Fig. 5c), which would ensure traceability of the quantitative omics. By using the reliable omics procedures, the real inter-sample differences can be captured, and efficient biomarkers might be discovered. To sum up, metrology thinking could have a role to play in reliable biological and multi-omics research, as the core of metrological mindset, ensuring traceability and defining uncertainty of measurements are undoubtedly the most effective solutions. The Quartet multi-omics reference material was highly recommended as a foundation for the research community to evaluate new technologies, labs, assays, products, lab operators, and computational algorithms [133]. Hence, the reference measurement procedures of multi-omics profiling would also be indispensable. Although metrological means and standardization methods of multi-omics profiling are still in their infancy, some NMI/DI communities, clinical laboratories, and genomics centers are already working to develop novel metrological capability, and reference measurement systems support the standardization of genomic or multi-omics studies. Therefore, it is time to establish an international working group to address the comparability issue of multi-omics measurements.

Conclusions

Science has endless frontiers while metrology being its solid foundation. Biometrology needs to keep pace with the increasing demands of life science and biotechnology to support the high-quality development of bioeconomy such as biomedicine, bioagriculture, and biosafety. Tools and method advancement push forward the measurement of increasing amount of real-world data, which will facilitate the development of biometrology. In the future, research and development of more biometrological standards and reference measurement procedures with scientific and application values as well as novel reference materials with multi-biological and multi-omics parameters are required and have to be developed. Better biological measurement results can best be achieved with the metrology mindset and practice.

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Declarations

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