News Release

International Standards Issued for Indirect Immunofluorescence Assays with Fluorescent Nanoparticles

A Joint Proposal by JMAC and Konica Minolta to Promote the Spread of Advanced Technology Originating in Japan

Tokyo (May 8, 2023) – Japan bio Measurement & Analysis Consortium (JMAC) and Konica Minolta, Inc. (Konica Minolta) announced that a proposal, driven by JMAC and jointly made with Konica Minolta, to establish international standards for performance evaluation requirements for quantifying biomolecules with fluorescent nanoparticles in immunohistochemistry was discussed by the International Organization for Standardization (ISO) technical committee for Nanotechnologies (ISO/TC 229) and has now been issued as ISO/TS 23366 *Nanotechnologies — Performance evaluation requirements for quantifying biomolecules using fluorescent nanoparticles in immunohistochemistry*.

Indirect immunofluorescence assays are a method of measuring the localization and quantity of a particular biomolecular species by staining cultured cells or microtomed tissue specimens with a primary antibody that recognizes the biomolecular species and a secondary antibody that recognizes the primary antibody. Assays of this kind include a technique in which a fluorescent substance is bound to a secondary antibody and a technique in which a low-molecular substance known as biotin is first bound to a secondary antibody, and the resulting conjugate is further reacted by binding a fluorescent substance to streptavidin, a protein that has high affinity for biotin. Assays are also used in clinical laboratory tests involving human sample staining, and in high-content screening for pharmaceutical development, and their markets are expanding.

While fluorescent dyes have traditionally been used to visualize the localization and quantity of a particular biomolecular species in indirect immunofluorescence assay, nanoparticles emit brighter fluorescence that fades much slower and are hence suitable not only for fluorescent imaging, but also for quantitative and other analyses. In particular, phosphor-integrated dots (PID) have been developed as fluorescent nanoparticles by Konica Minolta based on the company's technology honed through the development of particles for silver halide photography. PID has uniformly sized particle diameters at the nanometer level and higher luminance and lower fluorescence fading than conventional fluorescent dyes, thus enabling highly sensitive quantitative analyses that cannot be performed using conventional methods. With this feature, PID technology is used commercially in the Quanticell® services from Konica Minolta. Furthermore, the high luminance makes it possible to count bright spots from particles, and data have been obtained that demonstrate a correlation between the number of bright spots and the quantity of localized biomolecules. Accordingly, the company has proposed new quantitative methods, which are gradually spreading.

Developed jointly by the JMAC and Konica Minolta with the support of the Ministry of Economy, Trade and Industry*4, the newly issued standards cover indirect immunofluorescence assays with fluorescent nanoparticles used to label a secondary antibody or another molecule that binds to the secondary antibody, such as streptavidin, via biotin. The standards also describe basic requirements for nanoparticle-based indirect immunofluorescence assays, including the operating principle, nanoparticle choice, and quantification systems. The contents also include further requirements for data comparability, performance evaluation techniques, and common reference materials for mutual comparison of data analyzed using different systems, as well as validations, verifications, and reporting. As the requirements for quantification systems include quantitative methods based on the number of bright spots, the developed standard can be used as a base to show PID-related technology developed by Konica Minolta is internationally acceptable through conformity assessment.

The newly issued standards standardize performance evaluation indicators such as particle size, detection sensitivity, and quantification accuracy verification guidelines, for staining reagents, protein quantification kits, and other products, including Konica Minolta's PID. This achievement will facilitate the spread of immunohistochemical staining techniques based on fluorescent nanoparticles, including PID, in medical diagnosis fields, including pathological diagnoses. International standardization in medical diagnostic technology will assist the sharing of diagnosis results on a global scale, and also help establish the basis for increasing the global market share of Japan's medical industry and create a safe and secure society.

*1 ISO

The International Organization for Standardization, commonly referred to as ISO and headquartered in Geneva, Switzerland, is a non-governmental organization that formulates international standards in all industrial sectors except the electrical field.

*2 Quanticell

The highly sensitive quantitative analyses provided in the Quanticell® services employ PID technology based on high-luminance fluorescent nanoparticles that have been independently developed by Konica Minolta. Characterized by uniform particle diameters, higher brilliancy than those of fluorescent dyes and quantum dots, and high stability to excitation light exposure, the PID technique enables highly sensitive quantitative analyses that cannot be performed using conventional methods. The same immunostaining process as the conventional method is used, and thus special reagents or antibodies are not required because staining is done in stages in the order of primary antibody, secondary antibody, and PID.

*3 Name of the Japanese Committee for ISO/TC 229 and ISO/TC 229/WG 5
ISO/TC 229 Nanotechnologies, WG 5 Products and Applications Working Group

*4 These standards have been developed with the support of a strategic project for accelerating international standardization by the Ministry of Economy, Trade and Industry (2018-2020).

Konica Minolta, Inc.

Founded in 1873, Konica Minolta marks its 150th year in 2023. Konica Minolta has been creating new value in response to people's hopes and desires to "see." Its businesses were founded to capture the moment in the world as it is with tangible images by photographic films and cameras. Now, through visualizing technology and solutions that capture what

was once invisible to human eyes, Konica Minolta takes signs of illness and subtle variations in the quality of manufacturing. Konica Minolta continues to deliver innovation by anticipating trends in the business environment, from changes in remote work to the manufacturing process within various industries and business categories so that the company contributes to efforts to address challenges faced by customers and society. Konica Minolta positions the 150th milestone year as a new start to further leverage the power of imaging in driving sustainable growth for people and society.

Konica Minolta globally provides products and services in 150 countries, with Digital Workplace, Professional Print, Healthcare and Industry businesses.

Non-profit Organization Japan bio Measurement & Analysis Consortium

The Japan bio Measurement & Analysis Consortium (JMAC), initially named MicroArray Consortium, was founded on October 19, 2007 to promote industrial activities for microarrays and other biochips and develop their market and was approved by the Tokyo Metropolitan Government as a non-profit organization in the biotechnology industry in October 2008. As described above, the ISO 16578 International Standards for Biochips were issued in 2013. Thereafter, in October 2018, the organization changed its name to Japan bio Measurement & Analysis Consortium in line with its aim of promoting industrialization activities not only for biochips, but also for a broader range of biotech-related products. Since then, the organization has been engaged mainly in standardization activities.

Biotech-related technology is evolving rapidly and is widely used today as a highly useful research tool by universities and other academic organizations, as well as the research institutes of pharmaceutical, food, and other commercial firms. However, their industrial use, which is likely to become a much larger market than for academic use, is still limited due to the lack of technical standardizations, including methodologies and procedures for accuracy measurement, sample pretreatment, data analysis and assessments, reagent management, and other tasks.

Outside Japan, on the other hand, much work is being done on biotech-related technology standardization. With the increasing globalization of relevant markets, Japanese industry cannot ignore their impacts.

JMAC hopes that new markets for biotech-related technology will be created in Japan by standardizing biotech-related technology to assist its industrialization, and facilitating international harmonization with international organizations, including those in Europe and the United States, to promote standardization led by industry.

JMAC also expects that industrialization of biotech-related technology will be promoted by the exchange of information among biotech-related technology companies to identify and resolve issues hindering industrialization through various occasions, including workshops on patents, recommendation criteria, and other topics.

Accordingly, our consortium was founded to promote industrialization and create new markets through standardization of biotech-related technology.

For details on the consortium, visit our website at https://www.jmac.or.jp.

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