Instand-NGS4P – New Standardized Next Generation Sequencing Workflows for Diagnostics

From research and development to successful European standardization now on fast forward to international standardization - the CEN “Next Generation Sequencing” workflows are now available, on their way to ISO and will soon be open for participation again.

Molecular in vitro diagnostics have enabled significant progress in medicine while Next Generation Sequencing (NGS) takes a prominent place in the arsenal of molecular techniques used for diagnostics. NGS facilitates sequence analysis of nucleic acids that can result in more precise information useful not only to complete the histopathological diagnosis but also to address newer targeted therapies active on specific genomic mutation or translocation. Moreover, NGS is fundamental to understanding the resistance mechanisms escape to targeted agents and may guide therapeutic strategies on refractory tumors. However, NGS technologies come with complex workflows containing many steps and the need for analyte- and sample-specific processes (e.g., for different source material in different quantity or heterogeneity) as profiles of isolated DNA/RNA or methylated DNA can change during collection, transport, storage, and processing. Such changes in combination with the many critical choices that need to be taken during the complex workflow can make the diagnostic as well as research outcome unreliable or even impossible because the subsequent analytical assay will not determine the situation in the patient, but can result in an artificial profile generated during the pre-examination process and subsequent analysis.

Project “Instand-NGS4P”

To tackle these hurdles and better facilitate successful and reliable sequencing results, the 65-month Pre-Commercial Procurement (PCP) project “Integrated and Standardized NGS Workflows for Personalised Therapy”, in short Instand-NGS4P (1), was established. The project started in January 2020 and brings together 7 leading medical centres with extensive experience in the use of various NGS platforms in research and routine diagnostics as well as European patient representatives, a standardisation organisation (DIN) and partners participating in the European infrastructures BBMRI-ERIC, ELIXIR and several NGS related EU programs.

The objective of Instand-NGS4P is to develop, in close collaboration with manufacturers new innovative products for sample-specific NGS workflows by an elaborate tendering process consisting of three different phases. The developed products will support the NGS workflow from sample pre-analysis to medical decision-making, driven by patient and clinical needs, while also addressing regulatory requirements for in vitro diagnostics and referring to international/European standards and requirements concerning the development of reference materials and the implementation of External Quality Assurance (EQA) schemes for the entire NGS workflow. In the end, this PCP project will provide two fully integrated, standardized NGS workflows for the routine diagnosis of common and rare solid cancers from adult to pediatric.

New CEN Technical Specifications for NGS Workflows

While Instand-NGS4P is still pursuing the NGS workflow development, a huge goal for standardization has been achieved. Through DIN’s support in transferring relevant research results and information into the work program of the European committee CEN/TC 140 "In vitro diagnostic medical devices" as a new project proposal and managing the development of two resulting standardization projects with EMC’s (2)

leadership, the consortium has accomplished the publication of two CEN/TS documents in November 2023, with European national translations following in early to mid-2024:

- CEN/TS 17981-1:2023, *In vitro diagnostic Next Generation Sequencing (NGS) workflows — Part 1: Human DNA examination*
- CEN/TS 17981-2:2023, *In vitro diagnostic Next Generation Sequencing (NGS) workflows — Part 2: Human RNA examination*

Both CEN/TS documents contain analyte- and sample-specific NGS workflows for diagnostics of DNA/RNA from tissues, blood and body fluids from the pre-examination phase (sample collection), over the examination phase (library preparation, sequencing), to the post-examination phase (analysis and reporting) and are the first of their kind to encompass all the steps that are needed to obtain successful and reliable sequence results. Furthermore, both documents explain and apply the views on regulatory issues from the Regulation (EU) 2017/746 on In vitro Diagnostic Medical Devices (IVDR) and U.S. Food and Drug Administration (FDA) concerning NGS diagnostic quality issues. Moreover, they combine with and add to horizontal standards like ISO 15189 for medical laboratories or ISO 13485 for quality management system requirements for regulatory purposes in the context of medical devices. The CEN/TS documents also contain links to other existing NGS-relevant standards indicating their applicability along each included NGS workflow and were developed by coordinating with NGS-relevant activities in ISO/TC 276 "Biotechnology", ISO/TC 212 "Clinical laboratory testing and in vitro diagnostic test systems" and ISO/TC 215 "Health informatics" to facilitate broad application.

Future Aim – International Standardization

As the PCP project is still evolving and further results are created, the consortium is aiming for more - with the ultimate goal being the international standardization of NGS workflows. A first step in this direction has been reached with the circulation of both CEN/TS documents as new work item proposals within ISO/TC 212 for the development of EN ISO standards. The decision on the approval is anticipated for late October 2024.

If you are interested in joining the EN ISO standards' development within the International Standardization Organization (ISO), please contact your national standardization body for assistance. For German experts, DIN is happy to assist you through Björn Hermes (björn.hermes@din.de).

Instand-NGS4P will continue to provide NGS research results as standardization inputs to ISO/TC 212 to improve patient care. For further information about the Instand-NGS4P project, you can follow this link: http://www.instandngs4p.eu/

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(2) Medical University Graz, University of Florence, ERASMUS University Medical Centre (EMC), University of Milano-Bicocca, University Clinics of Schleswig-Holstein, St. Anna Kinderkrebsforschung, Centre Leon Bérard, Italian Federation of Cancer Patient Associations, European Cancer Patient Coalition, Deutsches Institut für Normung e.V. (DIN), Technical University of Munich, University of Ljubljana, University of Manchester, University of Liverpool, Organisation of European Cancer Institutes, University of Helsinki, BioXPedia and the International Prevention Research Institute.