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In vitro diagnostic Next Generation Sequencing (NGS) workflows

Part 2: Human RNA examination

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National foreword

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European foreword

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Introduction

Molecular *in vitro* diagnostics has enabled significant progress in medicine. Further progress is expected by new technologies analysing profiles of nucleic acids, proteins, and metabolites in human tissues and body fluids. Next Generation Sequencing (NGS) takes a prominent place in the series of molecular techniques used for diagnostics. It facilitates sequence analysis of nucleic acids that can result in precise information for diagnosis and progression of diseases.

The NGS technique, however, has a very complex workflow that contains many steps. The target nucleic acids can originate from different sources, e.g. tissues, blood, and body fluids. The profiles of the isolated RNA can change during specimen collection, transport, storage and processing (e.g. formalin fixation) making the outcome from diagnostics or research unreliable or even impossible because the subsequent analytical assay will not determine the situation in the patient but an artificial profile generated during the pre-examination process. The available material can be small, the cells in a tissue can be dispersed heterogeneously (e.g. ratio of tumour to normal), the target nucleic acids can be circulating in blood or body fluids free of cells or in circulating cells (e.g. circulating tumour cells (CTCs)). For a successful and reliable sequence result, a suitable strategy needs to be chosen for every case depending on the available material and disease conditions. Therefore, the NGS workflow can differ from case to case, and the NGS workflow steps need to be carefully considered and chosen to get a sound and reliable result to determine the best available treatment for the patient. In addition, sequence platforms can differ in their technique (e.g. detection of a change in a current or fluorescence) and approach (e.g. whole transcriptome, panels, short-read sequencing, long-read sequencing) for sequence assessment. The bioinformatics analysis can differ in approach and ability to detect non-conformities and unreliable sequence results. To enable such capabilities, NGS metadata needs to be collected during all workflow steps from the patient to the reporting. In addition, controls and added controls need to be analysed properly. This way non-conformities or detected unreliabilities can be reported to the patient and the treating physician. The reporting of diagnostic NGS results can differ in clarity and depth, which can lead to different interpretations.

Standardization of the entire NGS workflow from specimen collection to the reporting of the results to the patient and the treating physician is needed for the development of reliable NGS examinations.

This document draws upon previous work to standardize the steps for NGS examinations from tissues, blood and body fluids in what is referred to as the pre-examination phase (sample collection), the examination phase (library preparation, sequencing), and the post-examination phase (analysis and reporting).

In this document, the following verbal forms are used:

— “shall” indicates a requirement;

— “should” indicates a recommendation;

— “may” indicates a permission;

— “can” indicates a possibility or a capability.

1 Scope

This document specifies requirements and gives recommendations for next generation sequencing (NGS) workflows for *in vitro* diagnostics and biomedical research. This document covers the pre-examination processes, human RNA isolation, sequencing library preparation, sequencing, sequence analysis and reporting of the examination of sequences for diagnostic purposes from isolated RNA from, e.g. formalin-fixed and paraffin embedded tissues, fresh frozen tissues, fine needle aspirates (FNA), whole blood, circulating tumour cells (CTCs), exosomes and other extracellular vesicles, and circulating cell free RNA from plasma.

NOTE 1 Typical applications include, but are not limited to, NGS for oncology and clinical genetics, certain single-cell analyses.

This document is applicable to molecular *in vitro* diagnostic examinations including laboratory developed tests performed by medical laboratories, molecular pathology laboratories and molecular genetic laboratories. This document is also applicable to laboratory customers, *in vitro* diagnostics developers and manufacturers, biobanks, institutions, and organisations performing biomedical research.

This document is not applicable for in situ sequencing, forensic sequencing, sequencing of pathogens or microorganisms and microbiome analysis.

NOTE 2 International, national or regional regulations or requirements or multiples of them can also apply to specific topics covered in this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CEN/TS 17390-1, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for circulating tumor cells (CTCs) in venous whole blood — Part 1: Isolated RNA*

CEN/TS 17688-1:2021, *Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for Fine Needle Aspirates (FNAs) — Part 1: Isolated cellular RNA*

CEN/TS 17747, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for exosomes and other extracellular vesicles in venous whole blood — DNA, RNA and proteins*

CEN/TS 17742, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for venous whole blood — Isolated circulating cell free RNA from plasma*

EN ISO 15189:2022, *Medical laboratories — Requirements for quality and competence (ISO 15189:2022)*

EN ISO/IEC 17020:2012, *Conformity assessment — Requirements for the operation of various types of bodies performing inspection (ISO/IEC 17020:2012)*

EN ISO/IEC 17025:2017, *General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017)*

EN ISO 20166-1:2018, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue — Part 1: Isolated RNA (ISO 20166-1:2018)*

EN ISO 20184-1:2018, *Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for frozen tissue — Part 1: Isolated RNA (ISO 20184-1:2018)*