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In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids —

Part 3: Interpretation and reports

Dispositifs médicaux de diagnostic in vitro — Tests moléculaires multiplex pour les acides nucléiques —

Partie 3: Interprétation et rapports

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 212, *Medical laboratories and in vitro diagnostic systems.*

A list of all parts in the ISO 21474 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Introduction

The first generation of in vitro diagnostic (IVD) medical devices for nucleic acid-based molecular tests has been focused on detection or quantitation of a single nucleic acid sequence (e.g. viral RNA, mRNA, or genomic DNA) within a clinical specimen. By comparison, a multiplex molecular test simultaneously measures multiple nucleic acid sequences of interest in a single reaction tube or a system. The development and clinical use of multiplex IVD medical devices are rapidly expanding with the technological advances and new elucidation of the clinical significance of many biomarkers.

In comparison to single target analysis, multiplex molecular tests require an increased number of controls, more complex performance evaluation/data analysis algorithms, and more complex interpretation and reporting of results.[1,2] Some multiplex systems amplify multiple targets in a single reaction step and then split these into reactions for specific target detection.[3]

Laboratories can develop assays in-house ("laboratory-developed test (LDT)", "home-brew", or "in-house test") or use commercially available multiplex assays involving a variety of technologies and instrument platforms. Multiplex molecular testing provides large amounts of complicated and multifarious genetic information, resulting in significant challenges to the laboratory with regards to appropriate data analysis, interpretation and reporting.

Implementation of a multiplex molecular test identifies large numbers of genetic variations in a sample, which is crucial for optimal patient care, and treatment guidelines are developed based on specific molecular findings; therefore, it is imperative to standardize the interpretation and reporting of molecular results among laboratories performing these tests.

This document describes the requirements and recommendations for various aspects of interpretation and reporting of the results by multiplex molecular tests in order to ensure the quality of laboratory services of such tests, in implementing multiplex molecular nucleic acid tests for clinical use.

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In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids —

Part 3: Interpretation and reports

1 Scope

This document gives the general requirements for interpretation and reporting of multiplex molecular tests which simultaneously identify two or more nucleic acid target sequences of interest. This document is applicable to all multiplex methods used for examination using in vitro diagnostic (IVD) medical devices and laboratory developed tests (LDTs). It provides information for both qualitative and quantitative detection of nucleic acid target sequences.

This document is intended as guidance for multiplex examinations that detect or quantify human nucleic acid target sequences and microbial pathogen nucleic acid target sequences from human clinical specimens.

This document is applicable to any molecular IVD examination performed by medical laboratories. It is also intended to be used by laboratory customers, IVD developers and manufacturers, biobanks, institutions, commercial organizations performing biomedical research, and regulatory authorities. This document is not applicable to metagenomic massive parallel sequencing (MPS), but it is applicable to multiplex molecular methods including 16S sequencing.

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.

ISO 15189, *Medical laboratories — Requirements for quality and competence*

ISO 21474-1, *In vitro diagnostic medical devices — Multiplex molecular testing for nucleic acids — Part 1: Terminology and general requirements for nucleic acid quality evaluation*

3 Terms and definitions

For the purposes of this document, terms and definitions given in ISO 21474-1 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
process step
part of a process which is predominantly self-sufficient and consists of one or several unit operations

[SOURCE: ISO 10209:2022, 3.1.65]

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4 General requirements

Multiplex molecular tests are IVD and medical devices that measure multiple nucleic acid sequences simultaneously, such as multiplex PCR, DNA microarray, and MPS-based methodologies.

A multivariable molecular test is a molecular test that combines the values of multiple variables using an interpretation function to yield a single patient-specific result including “classification”, “score” and/or “index”. This is usually based on a platform of multiplex molecular tests, e.g. a miRNA assay.[5] For more guidance, see Annex B.

An increasing number of clinical and commercial laboratories have been performing multiplex molecular tests and issuing corresponding clinical reports to provide information for the care of their patients. However, the detected variants and relevant information in each report can differ because of the use of different methodologies (e.g. multiplex PCR, DNA microarray, and MPS-based), panels (e.g. commercial panels or laboratory-developed test panels), target enrichment strategies (e.g. targeted capture or multiplex PCR), sequencing platforms, improvement countermeasures, bioinformatics analysis processes, and databases (e.g. public databases or self-built databases) by different laboratories.

Based on accurate results of testing, laboratories shall make evidence-based testing interpretation and release accurate and comprehensive reports, to ensure the best diagnosis and treatment strategies for patients.

NOTE Further guidance on MPS is given in ISO 20397-2.

5 Interpretation of results

5.1 General

The interpretation method shall be fit for purpose and should be supported by a relevant validation study.

The laboratory shall have documented procedures for interpretation and reporting of results, including algorithms, software, and databases. Procedures for interpretation shall include measures to minimize the risk of cognitive bias.

For the implementation of quality management in the process of interpretation, see ISO/IEC/IEEE 90003, which provides guidance for organizations in the application of ISO 9001 to the acquisition, supply, development, operation and maintenance of computer software, and related support services.

Results by multiplex molecular tests, e.g. detected variants and combined values of multiple variables, should be carefully reviewed by appropriately trained molecular diagnostic professionals in the context of each complete case, including histological and clinical findings.

Evidence-based categorization, e.g. “classification”, “scoring” and/or “indexing”, shall be performed before reporting. Genomics is a rapidly evolving field; therefore, the clinical significance of any variant in therapy, diagnosis, or prognosis should be re-evaluated on an ongoing basis.

5.2 Methods for interpretation of results

Methods to analyse the test results can differ, depending on the intended use of the test and whether the test results are qualitative or quantitative in nature.

Multiplex molecular tests, such as DNA microarray, and MPS-based methodologies comprise wet analysis and bioinformatic processes. Bioinformatic processes should include considerations on genomic databases, reference sequence databases, variant identification annotation and categorization, and curation.

In interpretation of results of multiplex molecular tests, the laboratory should take it into consideration that the positive predictive value (PPV) and negative predictive value (NPV) of each target of detection is influenced by the prevalence of target diseases or conditions of interest.

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A multivariate assay with algorithmic analyses combines the results of two or more biomarkers, with or without patient demographics and clinical information, into an algorithm to generate a classifier to stratify patients into different outcome groups for subsequent clinical follow-up. The algorithm can be a simple linear regression model or more complicated non-linear model(s) when required.

Where a cut-off is applicable to an assay, the cut-off should be used to determine the clinical sensitivity and clinical specificity.

When the multi-variate assays, such as miRNA-based assays, are generated from multiple analytes with no diagnostic value for the individual analytes, the result should be described using the risk scores instead of using the reading from the individual analytes.

In case of multivariable molecular test (e.g. miRNA analysis), the algorithm integrates the expression levels of analytes and normalizes it into a single numerical score that classifies the individuals into positive, negative and in some cases, intermediate outcome groups. Since the inputs to algorithm are an individual analyte expression level or concentration, the validity of the algorithm shall be monitored.[2],[5] For more guidance, see Annex B.

As manual interpretation is prone to missing critical information generated by multiplex molecular testing, laboratories should put in place an automated procedure for the process of interpretation based on updating informative databases in a timely manner.

5.3 Documentation on bioinformatics analysis

The laboratory shall use a documented standard operating procedure (SOP) for bioinformatics to analyse, interpret, and report the results. A complete procedure manual shall be available on the workbench or in the work area.

The laboratory shall document all algorithms, software, and databases used in the analysis, interpretation, and reporting of results.

The versions of each of these components in the overall bioinformatics shall be recorded and traceable for each patient result.

For each component, the laboratory can use a baseline, default installation, or it can customize the process by using alternate configuration parameters in deploying individual bioinformatics tools or in running specific algorithms. These customized tools should be adopted to the extent that they do not affect the performance of the test and may be subject to additional verification and validation steps.

The laboratory shall document any customizations that vary from the specified configuration, namely which parameters, cut-offs, and values are used.

When describing the bioinformatics process, the laboratory should document the overall workflow of the data analysis and include the input and output files for each process step. For each step, the laboratory should develop and document acceptable quality control parameters for ensuring the specified performance characteristics.

Where applicable, the laboratory should develop and document criteria for variant calling and called parameters, including thresholds for read coverage depth, variant quality scores, and allelic read percentages.

Evidence of compliance with this document, i.e. ISO 21474-3, should be demonstrated with appropriate documentation.

The laboratory should also document the bioinformatics processes that are used for reducing a large data set to a list of either causal relation or candidate genes or variants or both. For example, in inherited disease assays, the laboratory should document approaches used to identify recessive (latent or occult), dominant (overt or explicit), and new variants.

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Where applicable, bioinformatics analyses are conducted by aligning sequence reads to a reference sequence. The reference sequence version number and assembly details shall also be identified. Further information is available in ISO 20397-2.

Variants shall be named according to international nomenclature used by sector organizations standards (e.g. The Human Genome Variation Society (HGVS), the Internal System for Human Cytogenetic Nomenclature (ISCN), and the International Union of Microbiological Societies)[1)], allowing explicit mapping to standardized reference numbers.

As the number of targets of interest increases in a multiplex assay, false negative (FN) results for certain sequences can become more problematic. In particular, the target with the lowest abundance within the nucleic acid sample should be assessed.

As the number of targets of interest increases, false positive (FP) results can become more problematic. For example, intrinsic limitation of microarrays is probe cross-hybridization to similar sequences within a genome. Sequence errors can also occur during nucleic acid amplification, leading to incorrect base calling. There is also a risk of FP results due to contamination while collecting and handling clinical specimens. Thus, the influence should be assessed with an appropriate method, such as using the quantitative measurement with cut-off values.

5.4 Monitoring of bioinformatics analysis

For bioinformatic analysis of generated data, the laboratory shall monitor validated performance of parameters, including robustness, accuracy, and reproducibility at each step.

5.5 Genomic databases

The genomic databases provide information that is necessary for accurate annotation and prioritization of variants. Laboratories should exercise the following cautionary steps on the use of public databases:

a) Understand the content of the database and how the data are aggregated. The laboratory should review the documentation or published literature relating to a given database to ascertain the source, type, and intent of the database.

b) Pay specific attention to the limitation of each database to avoid overinterpretation of annotation results.

c) Confirm the versions of the reference sequence version and assembly details as well as mRNA transcript references to ensure appropriate HGVS annotation or ISCN.

d) Whenever possible, use genomic coordinates, instead of HGVS nomenclature or ISCN, to unambiguously query genomic databases.

e) Assess the quality of the provided genomic data based on the source, from publications or another database, the number of a specific entries (single or multiple), the depth of the study, the use of appropriate controls, confirmation of a variant's somatic origin, and functional and potential drug response studies.

f) Verify data quality of the pathological diagnosis when provided (e.g. site, diagnosis, and subtype).

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1) The Human Genome Variation Society (HGVS)
https://hgvs-nomenclature.org/stable/ https://hgvs-nomenclature.org/stable/
The Internal System for Human Cytogenetic Nomenclature (ISCN)
https://iscn.karger.com
The International Union of Microbiological Societies
https://www.the-icsp.org/index.php/international-union-of-microbiological-societies

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NOTE 1 Public genomics resources include: University of California at Santa Cruz (UCSC) Genome Browser, ENSEMBL, DECIPHER, Database of Genomic Variants (DGV), Online Mendelian Inheritance in Man (OMIM), Genome Aggregation Database (gnomAD), The Human Gene Mutation Database (HGMD), ClinVar, Catalogue of Somatic Mutations in Cancer (COSMIC), etc. The genotype and phenotype database is available at National Center for Biotechnology Information (NCBI) and the Center for Genomic Epidemiology.[2)]

NOTE 2 Public genomics resources for pathogen genome data include: GenBank, EzBiocloud, KmerFinder, leBIBI, Type Strains Genome (gcType) Database, pubMLST, MycoBank, etc.[3)]

NOTE 3 Nextstrain is an open-source project to harness the scientific and public health potential of pathogen genome data.[4)]

5.6 Reference sequence databases

Reference sequence databases provide information on the version of the genome assembly and related information on human and pathogens, such as genomic coordinates, for unambiguous representation of sequence variants.

Species identification of pathogens can be performed on genome sequencing data by either 16S characterization, or by identifying short strings of DNA used in genome assembly (e.g. k-mer identification).

Particularly, in the case of miRNA, each sequence has its own nomination. The name and their sequences cannot always match each other since nucleic acid databases (e.g. miRbase) release is constantly being updated. The registered nomination should be referred to by the database name and the accession number.

5.7 Variant identification and annotation

Variant identification is a critical starting point of variant interpretation in human and pathogen genome. There are many variant detection software tools that cater to one specific alteration, such as single nucleotide variant (SNV), indels, structural variants, and copy number variations (CNVs). Laboratories shall understand the limitations of these variant detection tools. The laboratory should appropriately verify and validate the bioinformatic processes, including commercially purchased bioinformatics packages, to ensure the quality of the results.

One of the challenging aspects of variant annotation is the conversion of genomic coordinates (i.e. chromosome and position) to the corresponding cDNA/amino acid coordinate system (c. and p. syntax, respectively) for interpretation.

Certain metrics for detected variants should be included in variant evaluation for the interpretation. This is particularly important for somatic variant interpretation in the absence of paired normal and for evaluating tumor clonal diversity.

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2) UCSC Genome Browser https://genome.ucsc.edu
ENSEMBL https://asia.ensembl.org/index.html
DECIPHER https://www.deciphergenomics.org
Database of Genomic Variants (DGV) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3965079/
Online Mendelian Inheritance in Man (OMIM) https://www.omim.org
gnomAD (Genome Aggregation Database) https://gnomad.broadinstitute.org
The Human Gene Mutation Database (HGMD) https://www.hgmd.cf.ac.uk/ac/index.php
ClinVar https://www.ncbi.nlm.nih.gov/clinvar/
Catalogue of Somatic Mutations in Cancer (COSMIC)
https://www.sanger.ac.uk/group/cosmic-catalogue-of-somatic-mutations-in-cancer/
National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/

3) GenBank https://www.ncbi.nlm.nih.gov/genbank/
EzBiocloud https://www.ezbiocloud.net
KmerFinder https://www.genomicepidemiology.org
leBIBI https://bio.tools/lebibipqp
Type Strains Genome (gcType) Database https://gctype.wdcm.org
pubMLST https://pubmlst.org
MycoBank https://www.mycobank.org

4) Nextstrain https://nextstrain.org

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Special care should be taken when evaluating possible haematological malignancies because many commonly mutated genes in leukaemia and myelodysplastic syndromes can also be somatically mutated in the blood of otherwise healthy individuals (i.e. clonal haematopoiesis) and, therefore, can be incorrectly annotated as polymorphisms.

NOTE 1 In a multiplex molecular test, certain metrics for detected variants can be critical for variant interpretation, such as supporting reads (depth of coverage) and variant allele frequency (VAF).

NOTE 2 In chromosome microarray analysis, karyotype, gender, and other genetic information can be important for variant interpretation.

5.8 Categorization of variants

Each detected variant shall be categorized in an evidence-based approach. Variants include SNVs, indels, fusion genes resulting from genomic rearrangements, and CNVs. Interpretation of germline sequence variations should be focused on pathogenicity of a variant for a specific disease or disease causality. On the other hand, interpretation of somatic variants should be focused on their impact on clinical care. A variant can be considered a biomarker that affects clinical care if it predicts sensitivity, resistance, toxicity, prognosis or response to a specific therapy, or otherwise alters clinical decision-making because of its presence or absence.

In categorization of variants, the laboratory shall consider the requirements specific to the multiplex molecular tests in the entire workflow to ensure the accuracy of sequences, e.g. more stringency in quality and quantity of sample, an increased number of controls, and more complex performance evaluation/data analysis algorithms than singleplex variant detection. Detailed information can be found in ISO 21474-1 and ISO 21474-2.

The function of the gene, which can be targeted by approved or investigational drugs, serves as an inclusion criterion for clinical trials, influences disease prognosis, assists in establishing a diagnosis of disease (e.g. a cancer) or warrants implementing surveillance measures for early detection of disease. Clinical impacts should, therefore, include therapeutic, prognostic, diagnostic, and preventive actions. The clinical impact of a given variant should be determined according to currently available evidence. Evidence used for variant categorization can be weighed differently based on its significance in clinical decision making (see Annex A for additional information).

Determination of the pathogenicity or clinical impact of variants in disease remains a monumental task. Genomic alterations can have a spectrum of clinical utility, including diagnosis, prognosis, therapy selection, and monitoring of therapy. Peer-reviewed literature, clinical practice guidelines, and large-scale mutation databases remain primary resources for evidence needed to effectively assess clinical significance of a particular variant. The molecular professional should make an assessment regarding the evidence derived from these sources.

In the clinical microbiology laboratory, to deliver an actionable result, the processes include annotation, genome visualization and comparison, SNP/variant calling, and phylogenetic analysis. Clinical applications include detection of antimicrobial resistance, virulence determinants and multi-locus sequence typing.

NOTE 1 The interpretation of somatic mutations and recommendations of potential targeted drugs and trials are recommended by the joint recommendations of the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and College of American Pathologists(CAP).[10] 5)

NOTE 2 Clinical Genome Resource (ClinGen)'s gene curation process is designed to aid in evaluating the strength of a gene-disease relationship based on publicly available evidence.[11] 6)

NOTE 3 The National Center for Biotechnology Information (NCBI) has established a template to capture antimicrobial susceptibility phenotype information for those organisms submitted to the BioSample database.[13]

5) Association for Molecular Pathology (AMP) https://www.amp.org
American Society of Clinical Oncology (ASCO) https://www.asco.org
College of American Pathologists(CAP) https://www.cap.org

6) Clinical Genome Resource (ClinGen) https://clinicalgenome.org

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6 Reporting of test results

6.1 General

The reporting of test results is an essential part of multiplex molecular testing and should contain all the information required for the ordering physician and the patient to know what exactly was tested, what results were obtained from the test, and any additional pre-examination, examination, or post-examination factors that can influence the clinical interpretation of the results. In this context, what a test does not find (i.e. pertinent negatives or suboptimal signal) can be just as important, if not more so, than what a test does find.

In reporting of test results, the laboratory shall consider all the requirements specific to the multiplex molecular testing in the entire workflow, e.g. more stringency in quality and quantity of samples, an increased number of controls, more complex performance evaluation, data analysis algorithms, and more complex interpretation of results, than singleplex diagnostic testing.

An incomplete or unclear representation of the data can lead to clinical errors and incorrect patient management. Because comprehensive clinical reports are the basis for identifying best treatment strategies, clinical reports without appropriate clinical interpretations of genotypes and with insufficient medication information can make it difficult for clinicians to decipher the data and take appropriate action. Hence, evidence-based categorization of disease-related variants should be well documented in the reports. Medication-related information is susceptible to frequent changes and is the purview of the clinician interpreting the clinical report. If a report includes medication/treatment references, those references should be accurate.

Written reports should include results as well as specific content on parameters that have a direct impact on decision-making, such as sample quality (e.g. input DNA, QC metrics), the methodology, and assay performance elements, e.g. limit of detection for microarray platform, LODP (LOD).

The properties of the testing method [e.g. sensitivity, specificity, LODP (LOD), and minimal depth of sequencing coverage], and the amount of input DNA, are the basis of establishing FN or failed results.

The report should use internationally accepted terminology and standard nomenclature, such as using standard methods for describing nucleotide sequences. See 5.3 and 5.5.

In human genomes, additional information, such as accession and version for mRNA transcripts (e.g. NM_004333.4(BRAF):c.1799T>A (p.Val600Glu) and malignant melanoma) and exon boundary definition, is critically important for generating correct HGVS nomenclature for variants.

Reporting should clearly describe the test method and limitations. Clinical recommendations should be concise and correlate with histological and clinical findings, if applicable.

Reports should be static, and the date of issue should be clearly presented; they are not necessarily either recalled automatically or reissued, or both, when medical knowledge changes. Since medical knowledge does change rapidly, laboratories should anticipate being asked to reinterpret previous test results. Consideration should be given to developing a process for updating reports when specifically requested. The laboratory should consider adding a comment stating that the report reflects knowledge and policies in effect as of the date of the report and will not automatically update report without a specific request.

6.2 Reporting elements

Extensive analyses have been performed to stress the importance of accurate patient and sample identification and of using clear statements about testing results and comprehensive clinical interpretations.

These evaluation criteria involve five main components: patient identification, sample identification, testing interpretation, and methodological details, as well as evaluation on the detection accuracy, reporting integrity, and information sufficiency.

The assessment of detected variants is analysed based on the respective panel content, LODP (LOD), and intended results. Variants out of the specific detectable range should not be considered in the scoring process. FN and FP results, for which the reported genotype differs from the expected results, should be considered

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as critical errors because the clinical management would be affected. In addition, results for which a genotype has been reported in which it is below the stated LODP (LOD) (without additional verification or explanation) should be also taken as errors, because laboratories offering diagnostic mutational analysis of target (e.g. ctDNA) should test for the selected clinically relevant variants.

In addition to the detected variants, the report should also contain several other elements that can be relevant for more thorough analysis of the results or for comparison with other results obtained from this patient over time, such as the genomic coordinates, the genome build, and the transcript/pathogen reference sequence (e.g. PRJNA183844)[7], provided that this information does not detract from the ability of a patient and clinical provider to interpret the immediately relevant essential elements of the report. Along these lines, it can be advisable to include this information in a table format toward the end of the section or in another section with an extended description of results, away from the main results. VAF and coverage should be evaluated and included in the report when appropriate. The report should include the sequencing coverage cutoff for the assay used. All genes and hot spots, or both, not meeting the minimal required sequencing coverage criteria should be declared in the report as having failed.

Reports should not be limited to positive findings. Pertinent negatives should be reported, in a disease-specific manner. Pertinent negatives should be included for tier I drug/disease combinations, (e.g. the definitive lack of an epidermal growth factor receptor (EGFR) mutation in a patient with lung cancer or the definitive lack of a V-raf murine sarcoma viral oncogene homolog B (BRAF) mutation in a patient with melanoma), for the suspected pathogens, (e.g. *Salmonella enterica* in a patient with food-born enteritis). Uncertainty, if present, shall be communicated in reports. This includes issues of sequence quality, sample adequacy, tumor content, and biomedical knowledge.

6.3 Test report content

Test reports shall include information necessary for interpreting testing results (test reports should provide sufficient information to users, including appropriate medical interpretations with clinical significance).

The information necessary for interpreting the results of human genome sequence analysis includes the subject's clinical data, geoethnic ancestry, clinical sensitivity, and specificity of the specimen.

The test report shall be clearly stated so that the user can understand the clinical usefulness and limitations of the test results. If the quantity and quality of the specimen received can affect the results, it shall be stated in the report.

The following information should be included in the genetic testing report:

a) the need for genetic counselling by a qualified genetic counselling professional;

b) potential impact on family;

c) information on necessary additional testing.

The main subject of the report is genetic testing, but in some cases, nucleic acid tests for pathogens and molecular tests for somatic gene alterations are also assumed.

All information regarding the interpretation of the results should be attached to the final report, including when the testing was re-outsourced (referred to another or additional laboratory).

Laboratory shall define criteria for information on organisms that have a high clinical index of reportability, dependent on the intended purpose of the test. Pathogens should be distinguished from their near neighbours. Antimicrobial resistance genes phenotypic susceptibility data and virulence factors can be represented as subsets within existing databases.

Further information on test report content is described in ISO 15189. For more guidance, also see Annexes A, B and C.

7) NIH BioProject https://www.ncbi.nlm.nih.gov

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6.4 Reporting detected variants

It is useful to provide an interpretive comment on detected genetic alterations that places the alteration in clinicopathologic context to inform management decisions. This is essential for mutations with a strong or potential clinical significance (e.g. tiers I and II), as recommended as per a joint consensus of AMP and CAP (see Annex A for additional information). Detailed analyses of variants with unknown clinical significance (e.g. tier III variants), shall be balanced against the goal of keeping the most critical information in the reports concise, clear, and prominently presented. The comments may include functional, prognostic, or predictive significance of the variant for particular disease type, impact on biochemical pathway(s), and prevalence in relevant diseases.

Recommendations should be made, wherever possible, and defensible based on evidence, with appropriate literature citations. However, recommendations should be short and worded carefully, with the understanding that treatment or other patient management decisions are based on many pieces of medical information beyond genetic alterations, many of which are not available to the molecular professional issuing the report. Suitability for a treatment is based on many factors other than the diagnosis as written on a test requisition and the genotype or expression level of nucleic sequences discovered through testing. Often, these factors are unknown to the molecular professional reporting results (i.e. presence of confounding medical conditions, such as glucose intolerance, autoimmune disease, or heart failure), and failure to take these other factors into consideration when recommending a specific therapy can lead to confusion, conflict between patient and medical team, and anxiety. Treatment suggestions within the multiplex molecular laboratory report should be evidence-based, relevant to the patient's disease diagnosis, and should contain some kind of language to make it clear that the report contains generalized treatment suggestions incorporating the data points available to the laboratory (i.e. diagnosis and genotype) but those additional factors need to be incorporated into crafting a treatment plan for each individual. Recommendations for specific clinical trials should not be made, although general statements about availability of relevant trials or citing results of published trials are acceptable.

6.5 Reporting of secondary findings

Clinically significant genetic findings that are unrelated to the phenotype for testing can occur when performing target sequencing and whole genome sequencing. The laboratory should be aware of the potential for finding secondary clinically significant results and should have a policy in place for whether these results will be reported for those assays where such secondary findings are expected, e.g. exome.[14]

Laboratories can develop their own policies regarding the return of secondary results. If the laboratory's policy is not to report secondary findings or to limit reporting to a subset of variants related to a particular disease state, this should be clearly stated in the laboratory report for assays where secondary findings are expected. The test report should state that the policy on reporting secondary results is available on request.

Restricted sequence analysis of a panel of genes that are relevant to the diagnosis of a particular disease state (either with targeted sequencing or targeted bioinformatics analysis) can limit, but not eliminate, the potential for secondary findings. This can include identification of variants relevant to autosomal dominant (overt) diseases, carrier status for recessive (latent) diseases, predisposition to adult-onset dominant (overt) conditions (including cancer and neurodegenerative conditions), and drug response alleles commonly known as pharmacogenetic markers.

The American College of Medical Genetics (ACMG) recommendations for reporting medically actionable secondary findings include a minimum gene list for which if a known mutation is found, reporting is recommended. Laboratories can choose to follow the ACMG recommendations but are not necessarily expected to report only the findings of these genes.

Ethical considerations should also be taken into account when deciding whether to reveal certain genetic information to patients.

The level of risk associated with disclosing incidental findings depends on the severity of the disease, clinical actionability and other risk-benefit indicators. For example, common disease risk alleles, such as for type 2 diabetes or cardiovascular disease which have a small effect size (low relative risks) or pharmacogenetic risk information, can have different severity of consequences compared to the genetic information indicating

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a predisposition to cancer or a Mendelian disorder that can or cannot be medically treatable. All of these aspects shall be considered before returning the results to patients.

Concurrent analysis of a paired germline sample is desirable because it clarifies interpretation. However, it is not always practical and should not be required. When a paired germline sample is available, sequencing processes can allow separating germline findings from somatic acquired variants. Frequently, only somatic variants are interpreted and reported.

If germline variants are not reported in some of the genes in a multiplex molecular test panel, the initial report should specifically state that fact. If a patient or clinician requests additional analysis for germline findings, germline sequencing data can be rereviewed later and reported after appropriate patient consent is received. Consent, and documentation thereof, can be required for paired germline testing.

When paired germline samples are not used, multiplex molecular analysis does not distinguish germline and somatic variants, and sequencing results can contain both findings. In this case, findings can be reported with a disclaimer that the multiplex molecular test used does not allow definitive differentiation between germline and somatic variants. In certain settings, a germline variant can be suspected (e.g. VAF 40 % to 60 %). However, this interpretation should be made with caution and correlated with tumor cellularity. If a germline variant is suspected, testing of a patient germline sample (e.g. blood in patients with solid tumors) can be suggested. The reports should include a statement addressing the manner in which the distinction between somatic and germline alterations is made, and indications of remaining uncertainty, where appropriate.

If germline testing is ordered for cancer predisposition genes, reporting of germline variants should follow established guidelines, e.g. the ACMG/AMP guidelines. Genetic counselling and referral to a clinical medical geneticist should be offered. Laboratories should have policies regarding reporting of variants of unknown significance and disclosure of secondary findings, including under what circumstances such findings will or will not be reported.[10],[14]

Reference should be made to the description of the secondary genetic findings report “Proposals concerning the information transmission process in genomic medicine regarding comprehensive tumor genomic panel tests and germline whole genome / whole exome analysis”.

6.6 Reporting method

The test report should be able to convey the information in a way that is easy to interpret even for a non-healthcare professional. The report should be accurate, concise, and comprehensive, and should include all essential information so that the subject experts can make appropriate decisions.

Large panels (e.g. DNA microarray and MPS) sometimes need to convey large amounts of information, including technical elements about assay design. Some of this information is not of immediate use to all patients and clinical providers. The major findings in test reports should be short, simple, and to the point, and ancillary or reference information should not distract readers from understanding the report’s principal findings. All clinically critical information should be at the beginning of the report and formatted in a prominent manner to increase the likelihood that it is seen and understood by the physician. To increase the overall clarity of the report, provided that they can be integrated into the medical record, graphs, charts, and tables should be included. Methodological details should be presented at the bottom of the reports and should include a description of methods used, assay performance characteristics [especially LODP (LOD), minimal depth of sequencing coverage and minimal detection sensitivity of mosaicism], and critical quality metrics for the assay run. The report should include the details of what was actually tested. It is not sufficient for a report to simply list gene names unless the entirety of each of these genes was sequenced or an assay would detect all reported pathogenic mutations in the listed gene. The specific gene loci, exons, or hot spots tested should be listed in the final report. As gene panels become larger, including all this information in a report can become onerous. Laboratories can post additional information on a website that is available to all users. However, a stand-alone report is preferable.

The laboratory shall establish documented procedures for the release of examination results including details of who may release results and to whom according to ISO 15189.

Further information and guidance on reporting can be found in Annexes A, B and C.

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Annex A
(informative)

Multiplex molecular test for cancer

The interpretation and reporting of sequence variants in cancer has been recommended, as per a joint consensus of the Association for Molecular Pathology, the American Society of Clinical Oncology, and the College of American Pathologists.[10]

Clinical and experimental evidence has been proposed at four levels.

According to the joint consensus,[10] reports of somatic variants should indicate their category based on their clinical impact. For example, tier I variants have a strong clinical significance (level A and B evidence), tier II variants have potential clinical significance (level C or D evidence), tier III variants are of unknown clinical significance, and tier IV variants are benign or likely benign. Potential targeted drugs or trials should cite references and indicate their classifications (level A–level D).

Specimen type and sample quality are also included, as this can help to decrease the sample's negative effects and ensure the testing accuracy. A statement outlining the testing methodology and its limitations plays a vital role in determining the causes of false negative or false positive results and test failures.

a) Level A biomarkers are predictive of response or resistance to US Food and Drug Administration (FDA)-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors.

b) Level B biomarkers predict response or resistance to a therapy based on well-powered studies with expert consensus or have either diagnostic or prognostic significance, or both, of certain diseases based on well-powered studies.

c) Level C biomarkers predict response or resistance to therapies approved by the FDA or professional societies for a different tumor types (i.e. off-label use of a drug), serve as inclusion criteria for clinical trials, or have either diagnostic or prognostic significance, or both, based on the results of multiple small studies.

d) Level D biomarkers show plausible therapeutic significance based on preclinical studies or assist disease diagnosis and/or prognosis themselves, or along with other biomarkers based on small studies or multiple case reports without expert consensus.

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