

Concept Paper

## German Guidelines for Molecular Genetic Diagnostic Testing Using High-throughput Technology, Such As Next-Generation Sequencing

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**Academic Editors:** Ute Moog and Domenico Coviello

**Special Issue:** [Next Generation Sequencing](#)

*OBM Genetics*

2018, volume 2, issue 2

doi:10.21926/obm.genet.1802019

**Received:** September 24, 2017

**Accepted:** March 13, 2018

**Published:** April 28, 2018

### Abstract:

On behalf of the German Society of Human Genetics, we present guidelines for molecular genetic diagnostic testing using high-throughput technology, such as next-generation sequencing (NGS). These guidelines have been formulated by an expert group and reviewed by members of the German Society of Human Genetics. Building on the existing EuroGentest guidelines for diagnostic NGS, these updated guidelines incorporate additional aspects and



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country-specific topics. New considerations include the introduction of a check for the feasibility of a diagnostic test to protect patient interests, ensure precision and save resources. In addition, “diagnostic yield” has been replaced by “clinical diagnostic benefit” as a primary parameter for defining suitable test formats. Publicly available target gene lists for diagnostic interpretation and specification of the core genes, which should be the focus of most detailed analyses, have already been suggested. For German laboratories, the integration of cost-efficient and clinically-beneficial high-throughput diagnostic analyses designed to improve patient diagnoses is essential to fulfil current regulatory standards. The group endorses the majority of technical statements provided in the EuroGentest guidelines; however, regarding medical reports, a model of knowledge integration between the test laboratory and the ordering physician has been introduced in the updated guidelines to allow for more specific and better reporting in the individual medical context. Finally, a clear distinction between diagnostic testing and research-only analyses is proposed.

### **Keywords**

Guidelines; diagnostic next-generation sequencing; clinical genetic utility

## **1. Introduction**

The rapid adoption of next-generation sequencing (NGS) for molecular genetic testing and high-throughput diagnostic analysis in clinical practice has highlighted the need for German guidelines on best practice for its use. The scope of an expert-guided consensus on the specific implementation of this technology as a diagnostic tool extends beyond guidelines for using the technology itself and must include (specific) details on how the technology should be applied in a clinical diagnostic setting. Sanger sequencing has been applied for diagnostic purposes for more than 40 years and is still used to address a limited number of important clinical questions. NGS technology has only recently been transferred to patient care applications, and its longevity remains unclear in the light of emerging technologies, such as single-molecule sequencing, which are already under evaluation [1]. In recent years, the clinical application of NGS, in particular gene-panel sequencing, has been evaluated rigorously in parallel to addressing target enrichment [2], quality-related workflow elements [3], and the bioinformatics pipeline [4]. Clinical whole genome sequencing (cWGS) is now technically possible and eagerly anticipated as a high-quality clinical diagnostic tool; however, this technique has not yet captured much of the diagnostic market. This is due to the currently prohibitive cost of production of a 100x vertical coverage whole genome, although this technology is likely to be available for less than 300–400 EUR within the next 2–5 years.

With NGS platforms, products and production standards are still in development for clinical use; therefore, creating a formal set of guidelines for this rapidly evolving diagnostic application is extremely challenging. Nevertheless, there exists a need to provide useful guidance on important factors such as coverage, sensitivity and positive predictive value. Each of these parameters must be evaluated specifically and validated against the most up-to-date accepted thresholds, although any guidelines specifying detailed parameters could rapidly become obsolete with advances in the

technology. In light of the developing nature of the technology, an expert group has attempted to generate a revised set of guidelines, taking a broad approach to technical recommendations while collaborating with the members of the guideline group at the German Bundesärztekammer (Rili-BÄK expert group D5). These guidelines are designed to guarantee that important technical details can be defined and supervised within the required country-specific regulatory framework.

Diagnostic high-throughput sequencing has already been well-developed in groundbreaking work by Gargis et al. (2012) [5], Rehm et al. (2013) [6], Weiss et al. (2013) [7], and Matthijs et al. (2016) [8]. In addition, the highly regarded EuroGentest guidelines provide detailed guidance for laboratories working on the implementation of such technologies.

Nonetheless, there is still a need for further guidance to improve the application of high-throughput molecular genetic testing in clinical diagnosis that incorporates an up-to-date definition of the clinical diagnostic benefits and safety as well as the comparability of different test formats. It is also important to distinguish the application of these techniques for validated diagnostic testing as opposed to analysis for research-only purposes.

Here, we provide an English version of the approved German S1 guidelines explicitly for those statements that are not cited as such in other existing guidelines. The full German text can be found at the AWMF website ([www.awmf.de](http://www.awmf.de)) and will also be published in *Medizinische Genetik*.

## **2. Specific Statements**

### **2.1. Approving the Feasibility of a Requested High-Throughput Diagnostic Test**

#### **Statement 1: The test laboratory must approve the feasibility of the requested high-throughput diagnostic test**

Commentary: The application of high-throughput molecular tests is useful for the diagnosis of many rare diseases. Depending on the clinical question, the test indication is based on a formal genetic assessment, clinical observations, laboratory findings or other diagnostic reports. It is the referring physician's responsibility to assess whether or not a monogenic condition is suspected, or whether the test should be ordered to assist in the diagnosis. However, it is the responsibility of the test laboratory to evaluate the feasibility of the request and approve the test for use.

#### **Statement 2: Suitable indications for high-throughput testing should be decided based on the clinical findings and the proposed clinical diagnostic benefit**

Commentary: High-throughput testing permits the parallel analysis of multiple genes in a short time and at moderate cost, which significantly improves the time- and cost-efficiency and increases the diagnostic yield. The confirmation of a suspected diagnosis and the exclusion of differential diagnoses have an increasing impact on disease treatment and management. Incorrect clinical decisions for patients with a monogenic disease are a common issue. Examples include inappropriate surgery in patients with unrecognized hereditary spastic paraplegia or Marfan syndrome, prophylactic mastectomy after predictive identification of *BRCA* variants subsequently classified as sequence polymorphisms and erroneous anti-diabetic therapy in patients with maturity-onset diabetes of the young (MODY). Avoiding such clinical errors can be facilitated by high-throughput sequencing of whole genomes, whole exomes or multi-gene panels.

Compared to single-gene test, high-throughput sequencing can increase the analytical accuracy and power of evidence-based public data when identifying genetically heterogeneous diseases such as early childhood epilepsy, intellectual disability and cardiomyopathy. However, the diagnostic power of the data relies heavily on clinical inclusion criteria, technical specifications of the performed test, and the patient population tested. As a result, benchmark statistics for many rare and ultra-rare diseases are not available and can be very difficult to obtain. Without a consensus on the quality of the test or the test laboratory, crucial indicators will vary considerably between laboratories.

The co-testing of relatives of an index patient is a good example of the value of high-throughput screening. Co-testing is very helpful for the diagnosis of patients with intellectual disability as most of the pathogenic variants arise de-novo and are difficult to recognize without parental genotypes. Thus, the inclusion of parental samples for a trio analysis is pivotal for the diagnostic ability of the test.

**Statement 3: A high-throughput sequencing test requires a list of “target genes” that specifies the reportable range of the test**

Commentary: The selection of target genes for analysis requires careful evaluation of the most up-to-date data and should reflect guidelines and gene lists defined by expert group consensus. A gene list must only include genes with a clear, or very probable, disease association (as proposed by the ClinGen consortium [9]). In principle, this target gene list can be generated through specific DNA enrichment (targeted gene panels) or by bioinformatic filtering.

**Statement 4: The target gene list for high-throughput sequencing requires the designation of “core genes” (high-quality analysis, >99% coverage of the target region, so-called “class-A quality”)**

Commentary: This statement intends to promote market transparency and consistency. Moreover, a test-by-test assessment is required to confirm that all relevant targets (significance of non-coding mutations, repeat expansions, gene dosage, etc.) are included in the test design. Information on known test limitations and the need for additional non-high-throughput testing must be communicated by the laboratory in advance. An example of the generation of a diagnostic gene list with designated core genes is provided in the supplement of the AWMF document ([www.awmf.de](http://www.awmf.de)). Details on the definition of NGS quality standards have been published by Matthijs et al. (2016) [8].

**Statement 5: High-throughput genetic testing must be part of a stepwise diagnostic process taking into account the clinical and economic benefits**

Commentary: Genetic information from appropriate NGS can substantially shorten an expensive and lengthy diagnostic odyssey. The burden of the test has to be balanced with therapeutic and economic benefits as well as the wider benefits to the patient and their family. To avoid unsolicited results, the target region should be limited to the clinical question. As far as possible, a stepwise diagnostic process including NGS and other technologies such as array comparative genomic hybridization or fragment length analysis should be applied.

## **2.2. Technical Guidance for NGS-Based Diagnostics**

The expert group has reached a consensus regarding coverage of all relevant technical definitions by the current EuroGentest NGS guidelines [8]. Therefore, the German expert group endorses relevant parts of statements 01, 03, 04, 07–27, and 29 with the addition of country-specific explanations and comments in the German version (see supplementary information at [www.awmf.de](http://www.awmf.de)).

## **2.3. Interpretation of High-Throughput Testing**

**Statement 6: Where high-throughput analysis produces an ambiguous result, the potentially relevant variants should be discussed with the referring clinician in the context of clinical signs and family history before a final report can be issued**

In cases of ambiguity in the results of high-throughput molecular genetic diagnostic analysis, the results should be interpreted following two-step process. All relevant variants should first be classified and then interpreted in a second step within the given clinical context. Where possible, the latter step should engage the referring physician to discuss the best genotype–phenotype match in the relevant case.

## **2.4. Distinction Between Research-Only and Diagnostic Analysis Applications**

**Statement 7: Research-only tests must be labeled as such and are not permitted for diagnostic purposes without validation**

Commentary: A clear definition of high-throughput molecular genetic analysis for diagnostic purposes in contrast to research-only analyses is mandatory. Diagnostic tests are typically more expensive due to the validation and quality management required, whereas research tests are often funded only through research grants. Although non-validated research assays have the benefit of lower cost, diagnostic testing provides additional analytical value in terms of processing safety, robust bioinformatic validation, and a formal request and reporting format to ensure that results of diagnostic tests can be used directly in making clinical decisions.

## **Acknowledgments**

We thank the board of the German Society of Human Genetics (GfH e.V.), especially Thomas Eggermann and Uwe Kornak, for providing constructive comments.

## **Author Contributions**

PB drafted the German guideline and the manuscript. GW, DG, CMR, HB, HGK, UF, and UH edited and commented statements and edited the manuscript.

## **Funding**

The German Society of Human Genetics (GfH e.V.) supported meetings in Würzburg and Fulda for the discussion and harmonization of the guideline paper.

## Competing Interests

The authors have declared that no competing interests exist.

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