Practice guidelines for Targeted Next Generation Sequencing Analysis and Interpretation.

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1. INTRODUCTION

DNA sequencing is the most commonly used approach for mutation scanning and is widely regarded as the gold standard. Agreed practice guidelines for both the sequencing process and data analysis are important to achieve a high quality approach, with common quality standards across different laboratories. These guidelines do not constitute an experimental protocol or troubleshooting guide, rather they aim to establish consensus standards for identifying and reporting mutations. These guidelines refer to germline and not somatic testing.

Different standards will be required for clinical diagnostics than would be acceptable for a sequence-based research project, since results will stand for the lifetime of the individual and may have implications for relatives of the proband.

Next generation sequencing, also described as "second generation" or "massively parallel" sequencing has replaced Sanger sequencing as the primary methodology employed by researchers to identify novel disease genes. The ability to simultaneously analyse multiple genes ("gene panel") at a cheaper cost per base makes next generation sequencing an attractive solution for diagnostic testing of genetically heterogeneous disorders. Targeted next generation sequencing describes a strategy in which a specific set of genes related to the patient's phenotype are analysed within the context of a genetic "test". This approach aims to detect variants in all genes relevant to the patient's condition, while minimising the possibility of identifying highly penetrant mutations in genes unrelated to the patient's phenotype. In order to streamline laboratory workflows a broader spectrum of genes may actually be captured and

sequenced followed by the targeted analysis of “virtual” gene panels. Familial cancer panels containing a large number of known cancer predisposition genes as well as exome capture are two commonly used capture strategies for subsequent sub-panel analysis.

Targeted next generation sequencing services were introduced into the NHS in 2010, whilst the laboratory and analytical protocols are both varied and rapidly evolving, these guidelines aim to describe the general principles that underlie the quality requirements of this technology. This document considers quality aspects of the whole process of targeted next generation sequencing. It is essential that this process is carried out by appropriately qualified and experienced staff working within certified laboratories that are working to recognised international quality standards (such as ISO 17025 and 15189). If the process is part of a research project then the findings would be required to be confirmed in an appropriately certified laboratory.

Local sequencing practices may vary both in terms of the targeting strategy, sequencing chemistry/hardware, analysis software and reporting of results.

These guidelines are based on the principles established for Sanger sequencing (CMGS 2009 http://www.acgs.uk.com/media/774826/sequencingv2.pdf).They identify common elements for each part of the process and specify quality criteria that should be met or exceeded.

Guidelines are described as either:

- ***Essential*** practice which must be implemented to ensure quality of service.
- ***Recommended*** practice where more than one approach is satisfactory, however there is a clear advantage in following the advice given.

2. VALIDATION OF NEXT GENERATION SEQUENCING TESTS

It is essential to validate any new laboratory test. This includes validation of all aspects of the test process, i.e. targeting method, sequencing process and data analysis. The validation requirements will vary according to the methodology employed and the context of the clinical diagnostic application (Mattocks *et al*., 2010). It is important to understand the technical weaknesses of the methodology in order to ensure that these are assessed during the validation, for example the lower sensitivity for detecting insertions and deletions compared to single nucleotide variants (SNVs). For the purposes of validating test methodology the consequence of the variant with regard to its pathogenicity is not relevant, so it is appropriate to include benign variants in addition to disease-causing mutations.

2.1 Reproducibility

It is essential to derive information on reproducibility and robustness (particularly in terms of horizontal coverage) during the validation phase. It is recommended that validation samples are analysed from at least three independent sequence runs. Run-to-run comparisons will determine the level of multiplexing possible to ensure minimum diagnostic vertical coverage.

If the same test protocol is applied to multiple genes, then it is acceptable to perform the validation at the level of the process rather than the gene. Verification of gene panels on a smaller scale can subsequently be performed to check that equivalent results are obtained on a given panel compared to validation data. This should include analysis of read depth (vertical

coverage) across the targeted region, and sequencing of positive control samples when available. It is essential that the positive controls are representative of the disease mutation spectrum.

An ongoing measure of reproducibility should be incorporated into the laboratory procedure to maintain quality and highlight run-specific problems. For example quality scores per base or read depth should be monitored.

2.2 Sensitivity

Formal validation of sensitivity requires comparison of the test's performance with a 'gold standard' comprising the known, true results for a particular set of variants. These results should be determined using the best available alternative test or tests. For validation of NGS the gold standard is likely to be determined using Sanger sequencing, SNP genotyping assays, an orthogonal NGS method or some combination of these methods.

The power to determine sensitivity is directly related to the number of unique (different) variants used in the validation. It is important to note that variants used to optimize a test cannot then be used to validate that test since this will bias the results. The operator should perform the validation without prior knowledge of the variants present (i.e. the validation should be blinded).

The required sensitivity of the test will depend upon the clinical application. For example, replacing a Sanger sequencing test with a high pick-up rate may demand a higher sensitivity per gene than a large panel (10s to 100s of genes) in which the prior likelihood of finding a mutation is low and testing has not previously been available.

It is essential that laboratories are able to demonstrate 95% confidence that the error rate for heterozygote/homozygote mutation detection is no more than 5%. This requires concordant results for a minimum of 60 unique variants tested by the new method in an independent, blinded analysis and compared with the gold standard (Mattocks *et al*., 2010).

In reality, the technical sensitivity for detection of SNVs is likely to be significantly better than this. It is recommended that laboratories are able to demonstrate 95% confidence that the error rate for detection of heterozygote/homozygote SNVs is no more than 2%. See Appendix for recommended wording for a statement of sensitivity. This requires concordant results for at least 150 unique variants. To achieve this number of variants for validation it is acceptable to make use of samples with many characterised variants. For example commercial cell line samples are available that have many thousands of high confidence variant calls determined using a range of methods, for example the Genome in a Bottle Consortium (https://sites.stanford.edu/abms/giab). However, it should be noted that testing a single sample is not sufficient for formal validation, nor is testing only cell line samples. It is essential that the method is proven to function with the sample type that will be tested for diagnostic purposes.

For laboratories using common methodologies (i.e. targeting methodology and sequencing platform), evidence might be obtained by pooling data, as was previously done during the validation of unidirectional semi-automated Sanger sequence analysis by the CMGS (Ellard *et al*., 2009*)*. However, a multi-centre validation will be subject to variation in analysis pipelines between laboratories.

3. QUALITY ASPECTS OF THE LABORATORY PROCESS

3.1 Patient material

It is essential that suitable material for sequence analysis is available, that the proband has been correctly identified and that the appropriate clinical diagnosis and/or phenotype information is provided. The sample must be collected, identified, recorded and stored under quality controlled conditions appropriate for diagnostic testing. For example if a case has been recruited into a research project it may be necessary to collect an additional sample. Genomic DNA from peripheral white blood cells is the typical starting material. Alternative sources such as fixed tissue may raise quality control issues that are beyond the scope of these guidelines.

3.2 Targeting methodology

Targeted methodologies include PCR amplification (long range or standard amplicon), hybridization (liquid capture using RNA or DNA "baits") or methods developed specifically for next generation sequencing (e.g. Haloplex). Issues relating to specific methodologies are beyond the scope of these guidelines, but for PCR-based methods it is essential to check for primer binding site variants that could cause allelic drop out (CMGS 2009 http://www.acgs.uk.com/committees/quality-committee/best-practice-guidelines/).

It is essential to consider that some regions of the human genome are very difficult or impossible to sequence. These include:

- genes where the presence of pseudogenes makes unique targeting difficult
- highly GC rich regions
- repetitive elements

3.3 Template library preparation

Sample multiplexing is an integral part of next generation sequencing protocols and individual sample identification is obtained by "index tagging" or using "molecular barcodes". This refers to the process of adding a unique DNA sequence to each patient sample during the library preparation process in order that the sequences obtained from that patient sample can be extracted from the total sequence data. It is essential that there is a robust system (witness or barcode check) to ensure that the index tag recorded for the patient matches the index tag added during library preparation and when index sorting at the analysis stage.

Where custom index tags are used, it is essential to have more than 1 base pair difference (or edit distance) between tags to minimize the risk of errors during synthesis or sequencing that could generate two identical tags. An edit distance of at least 3 base pairs is recommended. Laboratories might also consider typing a set of SNPs by an alternative method to check that genotypes generated from the sequencing are concordant and confirm sample identity.

Any PCR steps within the template preparation require inclusion of a negative control to check for sample contamination.

3.4 Sequencing

A choice of sequencing chemistries/platforms is available, but issues relating to specific methodologies are beyond the scope of these guidelines.

3.5 Outsourcing

It is recommended that outsourced work only be performed by certified laboratories working to recognised international quality standards (such as ISO 17025 and 15189). The outsourcing laboratory may choose to prepare the sequencing libraries in-house, but outsource the sequencing to an accredited external provider. In this situation, the sample identity is defined by the index tag or molecular barcode incorporated at the library preparation stage and any sample mix-up in the outsourcing laboratory should not result in an incorrect test result for the patient, provided sufficient unique identifiers are available for use.

4. DATA ANALYSIS

4.1 Data quality and depth of coverage

Quality metrics are generated at multiple stages of the analytical process, for example quality scores associated with the base, read, alignment, variant call or strand bias. The acceptable thresholds should be determined during the validation process.

It is recommended that the following data quality markers are logged as part of the sequencing audit trail. These technical details are not routinely required on a clinical report, but in some circumstances it may be useful to include some of this data to help explain the results.

- Average base call quality scores for each position as a phred-like value for data to be used in analysis. Filtering or cutoff criteria for the exclusion of reads or bases from downstream analysis.
- Mapping quality scores if genome-wide alignment is performed.
- Number of reads mapped and the percentage of target covered at the minimum coverage required.
- The alignment algorithm and alignment settings (seed length, mismatch tolerance, mismatch penalties, gap penalties and gap extension penalties) should be recorded.

The minimum depth of coverage will depend upon the required sensitivity of the assay, the targeting/sequencing method and the type of mutation detected. The minimum read depth should be evidence based and will be established during the test validation process. It is important to note that ‘true’ read depth is a measure of the number of DNA molecules in the sample that are examined in the process. Any duplicate or identical reads could represent copies generated by amplification during the process and should be removed for the purpose of determining read depth. For hybridization-based targeting assays, ‘de-duplication’ is a standard component of analysis pipelines. However, for amplicon based assays this cannot be done and read depth is not a reliable measure of the number of starting molecules examined. Particular caution should be exercised when using low concentration or poor quality DNA (such as that extracted from FFPE sections). In these cases it is recommended that a proxy for read depth is determined during validation, for example, via a real time PCR amplification assay.

Regions of sequence not meeting the required read depth may be described within the report as low coverage or may not be required for the clinical report if a definite pathogenic mutation (or mutations) has already been identified. If the regions of sequence are clinically important then using other methods should be used, e.g. Sanger sequencing (especially important when replacing an existing Sanger test).

4.2 Defining the region of interest (ROI)

It is essential that the extent of the analysis is defined. A minimal region of horizontal coverage should include the coding regions of the gene and the invariant acceptor and donor splice sites. It is simplest in terms of utility for both the laboratory and the recipients of results if a standard distance from the intron-exon boundary is used for all genes in a panel. If this region needs to be extended or reduced for any reason this should be noted (e.g. due to technical limitations or a requirement to cover a particular known causative variant). At the time of writing approximately 92% of intronic single nucleotide variants listed as disease-causing mutations (class DM) in HGMD professional release 2015.3 fall within 10bp of coding exons (n=13,823). In this respect little is gained by extending the region of interest further into the introns. Consequently it is recommended that laboratories consider coding exon ±10bp as the minimal region of interest when designing new capture assays, but take into account the distribution of known mutations in a particular gene.

4.3 Analysis pipeline to identify variants

Software for data analysis may be supplied commercially or be open source. Most commercial programs cover the analytical process from read alignment to variant annotation, but in-house pipelines utilise different programs for read alignment, removal of duplicate reads, indel realignment, quality calibration, variant calling and annotation. Accurate versioning is essential and each software upgrade requires revalidation. There are multiple settings and options which again must be determined during the test validation. It is essential that all software or pipeline updates are verified to maintain equivalent or improved analysis standard. This may be achieved using an existing data and does not necessitate additional laboratory work.

Where different analysis pipelines are used, either because of software updates or for different analysis purposes, it is essential that analysis pipelines and/or their components are assigned version references and these are used to annotate all analyses. It is acceptable to permit adjustable analysis parameters or filters with a particular pipeline version, but it is essential that these are defined and that their settings are associated with all relevant analyses. It should be possible to determine the precise analysis tools used to generate all results.

4.4 Copy number analysis

For some NGS target enrichment methods (e.g. hybridization capture) it is possible to detect large deletions or duplications that would not be detected by Sanger sequencing or PCR-based NGS target enrichment. This is either because the variant spans one or both primer binding sites, cannot be amplified using the standard thermocycling parameters for the assay or cannot be amplified due to an incompatible primer configuration. As the majority of existing hybridization capture designs are focused on coding exons, laboratories typically perform copy number detection by comparative depth of coverage analysis between a test sample and batch of normalized controls. Genomic architecture affects probe hybridization at some loci (e.g. regions with high GC content); therefore the sensitivity of copy number variant (CNV) detection should not necessarily be assumed to be equal across all target regions (Taylor *et al*., 2015).

For comparative depth of coverage analysis, numerous open source and commercial software programs exist, all of which require validation before being used within the clinical diagnostic setting. This process will dictate whether normalization is performed using inter- or intra-batch control samples. If using inter-batch control samples, factors to consider include the expected lifespan of the probe set and whether it is necessary to perform regular protocol adjustments between batches. An inter-batch approach is often best suited to large targeted panels for which sequencer capacity restricts the number of samples that can be sequenced concurrently (e.g. exomes). If using intra-batch control samples it is helpful to know the mutation spectrum and

relatedness between samples; this insight will help prevent the masking of CNVs due to their occurrence in presumed normal controls. It is important to reduce experimental variation between samples; shearing and PCR thermocycling should therefore be performed consistently.

It is recognized that increased sequencer capacity (both total read count and read length) will lead to targeting strategies (e.g. whole gene capture) that are suited to alternative analytical methods. These include the identification of discordant read pairs or split read mapping across captured breakpoints. Ultimately, the diagnostic scenario will dictate the most appropriate bioinformatics approach to CNV detection. For example, homozygous autosomal and hemizygous X chromosome deletions can be detected at normally well-sequenced loci by observing an absence of mapped reads rather than employing a comparative read depth algorithm.

The size thresholds for the detection of indels by variant calling within reads or by copy number analysis have not yet been determined.

4.5 Annotation of variants

It is essential that variants are described in accordance with the Human Genome Variation Society (HGVS) recommendations (http://www.hgvs.org/mutnomen/). The reference sequence (with version) should be included on the report. It is recommended that the genomic coordinates with build number are also recorded, e.g. GRCh38. The HGVS guidelines recommend use of a LRG (Locus Reference Genomic sequence) if available for the gene of interest (see http://www.lrg-sequence.org/). LRGs aim to remain fixed to ensure standardization of nomenclature. In the absence of an LRG a coding DNA reference sequence may be used. The coding sequence should preferably be derived from the RefSeq database (http://www.ncbi.nlm.nih.gov/RefSeq/). The report should specify that the A of the translation initiation codon ATG is base 1. Mutalyzer software (http://www.humgen.nl/mutalyzer) can be used to check sequence variant nomenclature. The transcript version used to annotate the variant must be specified.

4.6 Filtering of variants

The filtering strategy to remove benign variants of no clinical significance will depend upon the likely mode of inheritance. For example, a heterozygous variant reported in an unaffected adult is unlikely to be the cause of a dominant, congenital disorder, but other heterozygous variants found in unaffected adults may be recessive mutations. It may be appropriate to filter out polymorphisms according to minor allele frequency, but threshold settings remain to be established and some recessive mutations are relatively common in certain populations.

There are multiple variant databases (e.g. Exome Aggregation Consortium, Exome Variants Server, 1000 genomes, dbSNP, HGMD, DECIPHER and in-house) which vary according to the population (including age, disease status, ethnicity), variant type, data quality and annotation (including pathogenicity status). An understanding of the structure and content of these databases is essential in order to utilize them effectively. In-house variant databases can easily be established by export of variant files (e.g. .vcf) to LOVD3 (http://www.lovd.nl/3.0/).

5. DATA STORAGE

Guidelines from the Royal College of Pathologists and the Institute of Biomedical Science recommend a timeframe for storage of data and records pertaining to pathology tests (current

document is edition 5, 2015). This guidance may be updated and if so current guidelines must be followed.

Storage of next generation sequence data challenges the existing guidelines since:

- It is not feasible to retain the raw image files due to their size
- Analysis methods are continually updated hence repeating the historical data analysis is unlikely to be possible and
- DNA samples are stored indefinitely so the raw material will be available for re-testing.
- It is essential to store the output file from the variant annotation step (e.g. vcf file) and some laboratories may choose to also retain the FASTQ or BAM files in order to re-analyse the read data in the future. These data should be stored together with a log of the informatics processing that was applied to the raw data in order to make the sequence and/or mapping files.

6. REPORTING

6.1 General principles

Reports should follow the general principles described in the ACGS reporting best practice guidelines (http://www.acgs.uk.com/media/949852/acgs_general_genetic_laboratory_reporting_recommendations_2015.pdf). Where possible reports should integrate sequence data with the clinical information that has been provided and variants should be annotated as described in 4.5 above.

Because of the established use of previous nomenclature, it is helpful for the common names to be referenced alongside the HGVS version. However the requirement to do this is diminishing with time as healthcare professionals have become more familiar with HGVS mutation nomenclature.

6.2 Methodology

The amount of information associated with next generation sequencing technology is vast, and too much detailed methodology in a clinical report could distract the reader from the key elements of the report. The report should convey sufficient concise information such that the reader understands the basic approach taken to generate the results (what has been sequenced), is assured that the results are reproducible (standardisation of variant nomenclature; confirmation of variants), and understands the limitations of the test (sensitivity; coverage; technical constraints).

The clinical report must give details of what has been sequenced. This is particularly relevant when no pathogenic mutations have been identified, so as the limitations of the test performed are obvious, but it is also important when mutations are identified because the clinician may need to know that additional mutations in other genes have not been found. This is more important for heterogeneous conditions where there may be an increased prior probability of a patient having causative variants in more than one gene. The report must therefore convey what clinical condition has been tested for, and indicate the gene(s) tested, using standardised HUGO gene abbreviations. Clearly when the number of genes being analysed is several hundred, thousands, or is the full exome/genome it is impractical to list all the genes, and the extent of the analysis must be described in a more appropriate way, e.g. use of a document controlled website.

It is essential to quote the transcript reference sequences (and version number) which have been used to annotate all variants detailed in the report, and it is recommended to quote reference sequences for *all* genes which have been sequenced (where this is practical for the number of genes analysed). The extent of intronic analysis must be given, i.e. exons +/- X base pairs, where X is the minimum number of intronic nucleotides that have been sequenced immediately flanking the exons. It is ambiguous to just say that splice sites have been sequenced (or similar wording), as there is no consensus on the region of interest, as discussed in 4.2.

The basic method used to enrich the target should be given, e.g. custom designed amplicon or hybridisation capture panel or commercial kit based approach, and the sequencing instrument used should be stated. More detailed information such as version numbers of in-house or commercial kits is not required in a clinical report since other more meaningful data such as the regions covered and limitations of the test will often be specific to different versions; details of versions of tests used must be recorded within the laboratory records however, following standard good laboratory practice.

Similarly, it is not necessary to indicate in the report all the *in silico* prediction tools and databases etc that have been used in the analysis of variants. However, it may be appropriate to refer to specific resources when describing the evidence for the interpretation of pathogenicity of a variant.

It is essential to describe the limitations of the test. This must include:

- The expected diagnostic yield if known (i.e. the proportion of cases with the phenotype in question in which a mutation is detected by the testing strategy that has been employed). As more heterogeneous diseases are being tested using gene panel based sequencing approaches, in many cases this information will not be available. Where the diagnostic yield is not known, it is recommended that patient results are stored in order to establish evidence for the diagnostic yield in the future.
- Horizontal coverage, given by the percentage of the region of interest (target) meeting the laboratory's minimum read depth, e.g. 99% of the target generated sequence at a minimum read depth of 20x. This must be given for the whole target (panel). It is also recommended to make this information available for individual genes, either in the report or in a separate technical report, or to say that the data is available via a web link, or upon request.
- The analytical sensitivity of the test defined by the read depth (vertical coverage).
- The spectrum of mutations that are detectable, or conversely the mutations which are not detectable; in particular it is essential to indicate whether exonic copy number variants and other types of large rearrangements can be detected or not. For some specific diseases it may be appropriate to indicate specific types of mutation that are known to be associated with the clinical condition and which cannot be detected, e.g. trinucleotide expansions, common inversions, low level mosaicism in different tissues, deep intronic mutations or the presence of pseudogenes which interfere with mapping of sequence and may reduce sensitivity.
- If horizontal coverage is incomplete and/or read depth does not meet the defined minimum it may be appropriate to recommend further testing depending on the clinical diagnosis.

If insufficient space is available within the clinical report, this information may be provided via a link to a document controlled website (and documented within the clinical report).

6.3 Mutation(s) detected

In the absence of a proven, robust tube transfer checking system (e.g. barcode scanning, witnessed transfers) or a secondary test (e.g. SNP assay) to assure sample identity at each stage, it is essential to confirm clinically actionable mutations included in a clinical report by an independent test from a new DNA dilution. This will provide additional data regarding test specificity (false positive rate) to support discontinuation of confirmatory testing once a tube transfer checking process or secondary identity test has been validated.

Class 4 and 5 variants (see Best practice guidelines for the interpretation of novel variants are available on the ACGS website http://www.acgs.uk.com/media/774853/evaluation_and_reporting_of_sequence_variants_bpgs_june_2013_-_finalpdf.pdf) should be confirmed as described above, whereas it is not essential to confirm variants of class 3 and below. While it is acceptable to not confirm class 3 variants initially, clinical reports may suggest that testing parents and/or other appropriate relatives could help to clarify the pathogenicity of such a variant. In these cases, laboratories can choose to confirm the variant either prior to suggesting testing of relatives, or at the time of testing those relatives. The choice should be based on how confident the laboratory is that the variant identified by NGS is real. It would be inadvisable to offer to test relatives for a variant which the laboratory has little confidence in and hasn't yet confirmed, whereas if the coverage and quality of the sequencing gives the laboratory high confidence that the variant is real it might be prudent to not confirm the variant initially as in many cases follow-up testing of relatives will not actually occur and thus resources will not be wasted unnecessarily.

The report should state clearly whether and how variants have been confirmed. Currently, the most common approach for confirming sequence variants relies on designing PCR primers to flank and amplify the site of the variant, followed by Sanger sequencing of the PCR product. If the results of Sanger sequencing (or alternative method of confirmation) demonstrate the presence of the variant in full accordance with the NGS data, and the flanking sequence data is unique and as expected from the reference sequence of the appropriate gene, it can be assumed that the variant is real. If the confirmatory test fails to detect a variant detected by NGS, the results of both tests should be re-assessed to look at the quality and possibilities of a false positive or false negative. Ideally if the NGS data revealed heterozygosity for a SNP in the vicinity of the sequence variant being confirmed, it may be possible to include the SNP locus in the PCR amplification, which would then offer an internal control for amplification and sequencing of both alleles in the Sanger sequencing assay. If this is not possible, it may be necessary to repeat the Sanger sequencing using different sets of amplification primers, or further investigations may be required to understand why different results were obtained.

It is recommended to include references for previously reported missense mutations, and splicing mutations outside the conserved splice donor/acceptor sites.

6.4 Variants of uncertain clinical significance

The pathogenic significance of missense or non-coding mutations is not always clear. Best practice guidelines for the interpretation of novel variants are available on the ACGS website (http://www.acgs.uk.com/media/774853/evaluation_and_reporting_of_sequence_variants_bpgs_june_2013_-_finalpdf.pdf).

Next generation sequencing tests for large gene panels may identify multiple variants of uncertain clinical significance. The prior probability of finding a pathogenic mutation in most genes within large panels will be lower than if just testing for the most common genetic causes.

Consequently it may be appropriate to set higher thresholds for follow up tests required to provide further evidence of pathogenicity. We recommend that variants of uncertain significance are only reported within the main body of the report if there is further genetic testing or clinical investigation that is likely to re-classify the variant as either likely pathogenic or likely benign. It is acceptable to report the remaining variants of uncertain significance in a separate "technical report", appendix, or to note within the report that this information is available on request. Benign variants or genetic risk susceptibility alleles should not be reported as this could lead to misinterpretation.

6.5 Submission of variants to databases

It is recommended that as a minimum, and assuming appropriate patient consent is in place, all mutations included in the clinical report are submitted to an appropriate freely accessible mutation database.

Complete upload of all variants (including polymorphisms) and associated phenotype information from every patient is the ultimate goal and software enhancements to facilitate automated data export from laboratory databases are under development.

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APPENDIX

To ensure sensitivity statements on reports or test descriptions impart accurate, useful and understandable information and that they can be easily compared between different tests and different testing laboratories, it is recommended that sensitivity is given in the following standard format.

The overall detection sensitivity for this test/gene panel is X% - Y% (95% confidence interval). This estimate is based on the following data derived from formal validation of the test and the current knowledge relating to the profile of pathogenic variations in this condition.

Variant type	Sensitivity of detection technology (95% confidence interval)	Known contribution to disease phenotype
SNVs		*Figures given here for variant types should sum to 100% (non-detectable variant types should not be included – e.g. CNVs)*
Indels		*If the profile of pathogenic variants is not specifically known this area can be used to give a reasonable estimate. Alternatively it could be stated that the spectrum is not known and give a brief description of how the overall sensitivity estimate was derived*.
Add additional lines for other variant types as appropriate		

[The following paragraph can be used by way of explanation, for example on a laboratory website, if deemed appropriate]

Validation of a suitable level of sensitivity requires large numbers of positive controls. It is often the case that appropriate numbers of suitable controls are not available for specific disease gene panels. Because of this most NGS panel tests will be validated at a technical level i.e. the overall sensitivity of the technology used will be determined using a range of representative positive controls that do not necessarily relate to the specific disease in question. It is known that NGS is less good at detecting insertion or deletion variations than single nucleotide variations. This means that, unless the profile of pathogenic variations is known for the condition and/or panel of genes, which is often not the case, it is not possible to define sensitivity accurately. For example the technical sensitivity for SNVs in a lab is 99% (95% CI) and that for indels it is 95% (95% CI). The sensitivity for a panel test where the predominant

pathogenic variations are indels could be significantly lower than that of a test where the predominant pathogenic variations are SNVs.

Example usage A

Where the validation covers the technology used in a test, but not the specific gene panel, and the mutational spectrum of the condition is not known.

The overall detection sensitivity for this test >95% (95% confidence interval).
This estimate is based on the following data derived from formal validation of the test and the current knowledge relating to the profile of pathogenic variations in this condition.

Variant type	Sensitivity of detection technology (95% confidence interval)	Known contribution to disease phenotype
SNVs	>99%	Not known. The overall detection sensitivity is a conservative estimate based on Indels being the predominant pathogenic variation in this condition.
Indels	>95%	

Example usage B

Where the validation covers the technology used in a test, but not the specific gene panel, and the mutational spectrum of the condition is known or the validation covers the specific gene panel tested (N.B. in this case it is important the spectrum of pathogenic variants is truly reflected in the validation)

The overall detection sensitivity for this test >97.8% (95% confidence interval).
This estimate is based on the following data derived from formal validation of the test and the current knowledge relating to the profile of pathogenic variations in this condition.

Variant type	Sensitivity of detection technology (95% confidence interval)	Known contribution to disease phenotype
SNVs	>99%	70%
Indels	>95%	30%