

Regulatory Guidelines for Registration of Next Generation Sequencing *In-vitro* Diagnostic Medical Devices (NGS IVDs)

Medical Devices Branch

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1. NGS based IVD tests intended to aid in diagnosis of individuals with suspected germline or somatic mutation-related diseases.
2. NGS based Tumour profiling IVD tests for screening / diagnosis of cancer, selection of patients for selective therapy and management (e.g. personalized medicine), or in disease staging.

The scope of this document does not include other NGS based IVD tests such as non-invasive prenatal test (NIPT), HLA typing tests, infectious diseases, etc. due to the differences in how some of the pre-clinical studies are performed.

This guideline presents the specific requirements applicable for NGS IVDs that are to be submitted for registration.

The format for Product Registration submission for all IVDs is described in the GN-18 Guidance on Preparation of a Product Registration Submission for IVD medical devices using the ASEAN CSDT.

1) Scope

2) Background

3) Risk Class

4) Information Required

NGS based tests are qualitative in vitro diagnostic tests that use targeted next generation sequencing, whole genome sequencing (WGS) or whole exome sequencing (WES) either intended to aid in diagnosis of individuals with suspected germline disease or somatic mutation-related disease, or in screening / diagnosis of cancer, screening for selection of patients for selective therapy (e.g., personalised medicine), treatment management (e.g., residual disease determination and risk of relapse), or in disease staging.

1) Scope

2) Background

3) Risk Class

4) Information Required

NGS based IVD tests for diagnosis of germline or somatic mutation-related diseases

- The term “germline diseases” encompasses those genetic diseases or other conditions arising from inherited or de novo germline variants. Examples of suspected germline diseases could include developmental delay, congenital dysmorphologies, or other clinical features.
- The term “somatic mutation-related diseases” encompasses other non-cancer genetic diseases that does not arise from a mutation in germ cells (e.g., somatic mosaicism).

1) Scope

2) Background

3) Risk Class

4) Information Required

BACKGROUND

NGS based tumour profiling IVD tests

Targeted next generation sequencing, whole genome sequencing (WGS) or whole exome sequencing (WES) of formalin-fixed paraffin-embedded tumour tissue (FFPE)/ fresh tumour tissue/ liquid biopsies from patients with malignant neoplasms can detect tumour specific gene alterations. These NGS based IVD tests can be intended to provide information on somatic mutations (point mutations, small insertions, deletions and gene fusions) and genomic signatures such as microsatellite instability (MSI) and tumour mutational burden (TMB) which can be used for screening / early diagnosis of cancer, identify patients who may benefit from treatment with the appropriate targeted therapies (e.g., personalised medicine), to provide prognosis, to detect residual disease and to determine risk of cancer relapses. This information can be used by qualified health care professionals in accordance with professional guidelines, to guide and manage future treatment procedures.

1) Scope

2) Background

3) Risk Class

4) Information Required

Majority of the NGS based IVD kits which are intended to aid in diagnosis of individuals with suspected germline disease and in screening for selection of patients for selective therapy and management, or for disease staging, or in the diagnosis of cancer (e.g., personalized medicine) are classified as Class C medical devices. Due consideration should be given to the clinical use of the tests when determining the risk class.

For more information on Risk classification of NGS based IVD test kits, please refer to Rule 3 of GN-14-Guidance on the Risk Classification of In Vitro Diagnostic Medical Devices.

1) Scope

2) Background

3) Risk Class

4) Information Required

- 1) Information on interrogated regions of the genome
- 2) Sample collection and processing
- 3) DNA/RNA extraction and quantification
- 4) Library construction
- 5) Sequencing and data analysis (Generation of sequence reads and base calling, sequence alignment/mapping, variant calling, variant annotation and filtering, variant evaluation and assertion)
- 6) Controls used in the IVD test
- 7) Test Report generation
- 8) Pre-clinical studies
- 9) Clinical studies



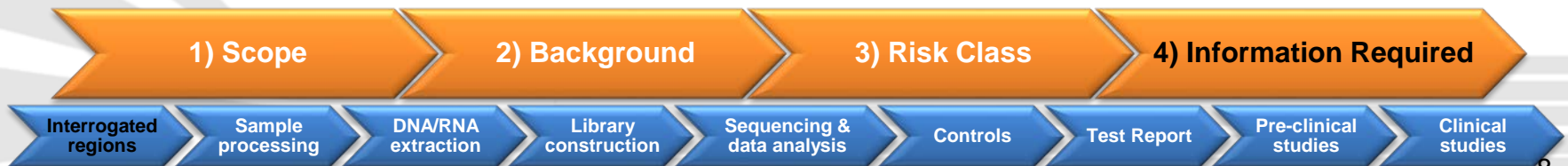
INFORMATION REQUIRED FOR NGS BASED IVDS

Information on interrogated regions of the genome

Interrogated regions of the genome (including genes and variants) should be specified and documented under “Device description and features” section of TR-02 Contents of a Product Registration Submission for In Vitro Diagnostic Medical Devices using the ASEAN CSDT document.

Evidence based assessment should be provided to justify the genes included for a given indication (e.g., clinical study, reference to literature).

Interrogated regions should meet minimum performance specifications (e.g., minimum coverage thresholds)



For NGS based IVD tests which are intended to aid in diagnosis of individuals with suspected germline disease:

- Acceptable specimen types to be used for the test should be specified and documented. Examples of specimen types include but are not limited to: whole blood, buccal swab. Genomic DNA (gDNA) is isolated from the appropriate specimen collected.

Additional factors to consider during sample collection and processing:

- Type of collection device required
- Minimum volume or quantity of sample, or any collection conditions that must be adhered to for sample stability between collection and use.
- Multiple specimen and collection types may be appropriate for a test (depending on the use of the test), but each type should be validated for use in producing DNA of the appropriate quality and quantity and for overall test performance.



INFORMATION REQUIRED FOR NGS BASED IVDS

Sample collection and processing

For NGS based IVD tests for tumour profiling:

Minimum requirements for tumour volume and tumour content/tumour fraction to obtain sufficient DNA/RNA for overall test performance should be specified and documented. A purely illustrative and simplified example is shown in the table below:

| Tissue Type | Volume | Minimum Tumour Proportion | Macrodissection requirements (based on tumour proportion) |
|---------------|---|---|--|
| FFPE sections | 5-20 unstained sections, 10 microns thick | <p>Confirm the tumour content of each sample based on the area of a haematoxylin and eosin (H&E) stained section.</p> <p>Provide prerequisite of tumour content. E.g., More than 10% of tumour cells; sections containing >20% viable tumour are preferred.</p> <p>For MSI testing, >25% tumour cells, etc.</p> | <p>If the tumour content is less than 20% and the tumour content in the region of interest is greater than or equal to 10%, Macrodissection and enrichment of the sample for tumour content is necessary. Following tumour enrichment, proceed with the extraction protocol.</p> <p>It is recommend to macrodissect highly necrotic areas or select alternate samples if possible.</p> |

Similar information should be provided for RNA (if applicable)



INFORMATION REQUIRED FOR NGS BASED IVDS

DNA/RNA extraction and quantification

- Minimum concentration of DNA/RNA required for the NGS based IVD assay to perform must be validated and stated clearly.

1) Scope

2) Background

3) Risk Class

4) Information Required

Interrogated regions

Sample processing

DNA/RNA extraction

Library construction

Sequencing & data analysis

Controls

Test Report

Pre-clinical studies

Clinical studies

Library preparation method should be validated as part of the IVD assay's workflow. Any subsequent changes to the library preparation method will require re-validation.

Library average fragment size and the library concentration should fall within the specific range stated in the respective library preparation kit used.

Examples of library preparation methods:

- Hybrid capture method
- Amplicon(PCR) based library preparation



- Sequencing instrument
- Generation of sequence reads and base calling
- Sequence alignment/Mapping
- Variant calling – SNVs and Indels
- Variant annotation and filtering - Summary of variant filtering scheme (work flow diagram) can be provided
- Copy number analysis (CNA) (if applicable)
- Microsatellite Instability (MSI) status calling (if applicable)
- Tumour mutational burden (TMB) status calling (if applicable)
- Mappable Fusion Reads and list of gene fusions detected by the IVD test (if applicable)



INFORMATION REQUIRED FOR NGS BASED IVDS

Controls used in the IVD test

- Information on appropriate internal process related to the IVD test controls used should be provided.

E.g.,

- Matched normal control (if applicable)
- Positive control
- Negative control
- Sensitivity control (If applicable)
- Extraction control



INFORMATION REQUIRED FOR NGS BASED IVDS

Test report generation

- Annotation software used
- Merging data with patient demographic information and any other additional information (If applicable)
- Presentation of results in report
- Clinical interpretation (if performed by the software)
- Summary of final approval process before report generation



Determination of bioinformatic pipeline thresholds

Analytical sensitivity

- Validation of sample input requirements
- Limit of detection (LoD)

Analytical specificity

- In silico specificity study (primer specificity study)
- Interfering substance study
- Cross-reactivity study

Precision/reproducibility

- Assay reproducibility study

Linearity/measuring Range (If applicable)

Traceability & expected values (controls, calibrators, methods)

Cut-off value (same approach as other IVDs)

- Microsatellite instability (MSI) score determination and cut off values.
- Tumour mutational burden (TMD) score determination and cut off values.

Trueness (not applicable)

Stability of reagent

Specimen stability

- Extracted DNA/RNA stability
- Genomic DNA/RNA freeze-thaw study

Carryover effect/cross-contamination

- Sample carryover study

Specimen matrix study

- Tissue comparability study
- Tissue fixation study (for FFPE samples)

1) Scope

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Determination of Bioinformatics pipeline thresholds

- Requirements on target region (e.g. exon, intron, promoter) coverage should be established
- Requirements on sample coverage
- Requirements on mutation coverage, uniformity of coverage, allele depth and frequency for positive calls
- Requirements on fusion gene detection (if applicable)



Analytical sensitivity

Validation of sample input requirements:

Sample input requirements for the assay to work as intended must be specified and validated. They may be validated as part of the workflow in other pre-clinical/clinical studies or as a study on its own. Below are examples of sample input requirements that may be applicable:

1) Primary sample input amount

The recommended primary sample input amount should be validated to determine if they would yield the required DNA/RNA amounts required for the assay when extracted using the specified DNA/RNA isolation protocol.



Analytical sensitivity

2) DNA/RNA Input:

The DNA/RNA input amount/purity requirements should be validated to meet the minimum amount specified in the IFU that is required for the IVD assay to perform as intended.

For example:

The assay performance can be tested across several DNA/RNA input concentrations. If the expected gDNA input for an assay is 250 ng, DNA samples can be tested at input levels of 1250, 250, 100, and 25 ng. The upper and lower bounds for DNA input should be determined. The study should demonstrate high percentage of positive and negative variant call rate (>95%) within the DNA input range tested, supporting the specified DNA input amount claimed by the NGS IVD Test kit. Samples with known somatic mutations/fusion genes from cell lines and clinical samples can be used for this validation purpose.

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Test Report

Pre-clinical studies

Clinical studies

Analytical sensitivity

3) Tumour content of primary samples (applicable – for tumour profiling IVDs)

The minimum tumour content in primary samples that is required for the assay to perform accurately.

For example:

Pre-characterized clinical samples (with established number of SNVs, deletions, and gene fusions confirmed by validated reference methods) can be used for tumour content validation process. The tumour cell content of each specimen and region of interest should be estimated before the study. Broad range of samples with varying tumour content percentage (<10% to 60-70%) should be included in this study.

For liquid biopsy samples, appropriate sample input amount requirements should be validated to meet the minimum specified tumour fraction requirement for the IVD assay to perform as intended.



Analytical sensitivity

Limit of Detection (LoD) study

For tumour profiling IVDs, the LoD is the lowest Allele frequency (AF) of single nucleotide variants (SNV), Multi-nucleotide polymorphisms (MNP), or deletion variants, or the lowest number of reads of RNA fusion variants that can be detected at least 95% of the time. Different test methods can be used to determine the LoD of the IVD assay.

1. Variant-containing samples can be blended with Wild type (WT) samples at multiple levels (serial dilution) and used as the input material for the test.
2. Confirmation of the LoD can also be done (Specific LoD of Variant Allele frequency (VAF) claimed by the test kit). For example, IVD test kit with LoD of 5% VAF claim, can test for 5 replicates of a sample for certain number of deletions, insertions and SNVs at 5% minor allele frequency. All variants ideally should have 100% positive call rates.
3. Commercially available reference standards can also be used for LoD validation purposes.

1) Scope

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Test Report

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Clinical studies

Analytical specificity

In silico specificity study

- An in silico cross-reactivity analysis needs to be performed that evaluates all the primers in the NGS IVD Test Kit to determine the specificity of the primers to their targeted sequences. Primers should be checked for specificity to the human genome, human transcriptome, and genomes from bacteria, fungi, and viruses frequently found in human specimens.
- Corresponding pseudogenes for any target gene on the NGS panel should be highlighted if they can potentially cause mismapping.

False positive rate in well characterised samples/cell lines

- To ensure that a variant-free ("blank") sample does not generate an analytical signal that might be classified as a mutation. Wild-type (WT) samples should be evaluated at each variant location that can be detected by the IVD NGS Test kit. Samples that are WT at all locations should produce a "variant not detected" call at each location. This tests provide information regarding the false positive rates.



Analytical specificity

Interfering substance study

- The effect of potential interfering substances on the performance of the assay should be examined.
- Examples of potentially interfering substances include bilirubin, hemoglobin, cholesterol, lipid, melanin, short draw interference, buffer component interference, paraffin, xylene, ethanol, protease and therapeutic drugs.
- Samples should be spiked with various potential interfering substances prior to nucleic acid extraction. For the assessment of each inhibiting substance, data for each spiked sample should be compared to the control samples. Impact on call rate, reproducibility, and concordance between variant calls in samples with and without interfering substances should be determined.

Cross-reactivity study

- Potential cross-reactivity to other organisms that may be present in sample type should be assessed.
- Potential cross-reactivity to other cancers or diseases should be assessed.



Precision/reproducibility

The study should be designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility).

The reproducibility and repeatability of variant/fusion gene detection using the NGS IVD Test kit can be assessed with WT samples and variant-positive samples. Each sample should be tested several times at different sites and total number of replicates tested per sample should be stated. Ideally, the study should include samples close to the LoD. The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability should be computed at each variant location of interest.

Additional tests that can be performed:

- Reagent Lot-to-Lot Reproducibility
- Instrument-to-Instrument Reproducibility
- Reagent Lot Interchangeability



Linearity/measuring range (If applicable)

Appropriate linearity/measuring range studies must be provided for assays that are quantitative, for example in residual disease determination and in disease staging.



Cut-off value

Microsatellite instability (MSI) validation (If /when applicable – for tumour profiling IVDs)

Precision of the MSI calling by any specific methods (e.g., MSIsensor) should be demonstrated with a total of (n) specimens which should contain both MSI-H (high) specimens and Microsatellite stable (MSS) specimens. Each DNA extracted sample can be tested with multiple inter- and intra-run replicates. Concordance score between the calls for all replicates tested should be summarised.

Precision and Reproducibility testing should include MSI performance as well.



Cut-off value

Tumour mutational burden (TMB) validation (If /when applicable – for tumour profiling IVDs)

- TMB score determination method should be provided in sequencing and analysis part.
- TMB concordance should be evaluated by comparing the TMB output in terms of mutations per Mb with appropriate control samples.
- Performance of TMB at the recommended tumour purity (e.g., $\geq 20\%$), should be validated on samples near the claimed TMB cut-off.
- Precision and Reproducibility testing should include TMB performance as well.

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Interrogated regions

Sample processing

DNA/RNA extraction

Library construction

Sequencing & data analysis

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Pre-clinical studies

Clinical studies

Stability of reagent

- Long term stability (3 lots of reagents required)
- In-use stability (if reagents are not single use)
- On-board stability (if applicable)
- Shipping studies

Where stability testing is performed on a representative panel, scientific justification must be provided.



Specimen stability (if there are specific claims)

Specimen stability claims may also be supported with specimen stability studies or with literature/guidelines.

Extracted DNA/RNA stability

Extracted DNA/RNA stability should include testing of extracted DNA/RNA stored at various temperatures/storage periods as claimed in the IFU of the NGS IVD kit.

For example:

- DNA extracted from anti-coagulated blood stored at room temperature (20-25°C) for 7 days
- DNA extracted from anti-coagulated blood stored at at 2°C to 4°C for 30 days
- DNA extracted from anti-coagulated blood stored at -15°C to -25°C for 30 days

Genomic DNA/RNA freeze-thaw study:

- The impact of repeated freeze-thaws on gDNA samples isolated should be evaluated.



Carryover effect/cross-contamination

Sample Carryover study

- The goal of this study is to ascertain that sample carryover between samples within an instrument run and between successive sequencing runs meets design requirements.

1) Scope

2) Background

3) Risk Class

4) Information Required

Interrogated regions

Sample processing

DNA/RNA extraction

Library construction

Sequencing & data analysis

Controls

Test Report

Pre-clinical studies

Clinical studies

Specimen matrix study

Tissue Comparability study (If /when applicable – for tumour profiling IVDs)

The goal of the study is to demonstrate that genomic profiling can be performed on DNA/RNA derived from different tissue type (as claimed by the assay).

It is also acceptable for the claims for different tissue type to be validated as part of the workflow in other pre-clinical/clinical studies.

Tissue fixation study for FFPE samples – (If /when applicable – for tumour profiling IVDs)

If formalin fixation time is a potential issue, potential effects of formalin fixation times on the assay performance should be addressed.

1) Scope

2) Background

3) Risk Class

4) Information Required

Interrogated regions

Sample processing

DNA/RNA extraction

Library construction

Sequencing & data analysis

Controls

Test Report

Pre-clinical studies

Clinical studies

- Accuracy testing of the NGS IVD kit should be assessed with well established methods (e.g, such as a validated NGS assay, sanger sequencing and/or FISH analysis). Reference variants from clinical specimens, HapMap cell lines and synthetic samples derived from plasmid DNA can be used as reference/control samples. Concordance between variant calls in samples processed between NGS IVD kit and reference method should be evaluated.
- For NGS IVD test kits with companion diagnostic claims, clinical trials should be conducted to support the companion diagnostic claims indicated in the intended use statement of the NGS IVD test kit. If the NGS IVD test kit is not validated together with the drug in a clinical trial, a method comparison with an established companion diagnostic IVD test/method using clinical samples representative of the intended use population may also be used. All genetic alterations which are in the companion diagnostic category level mentioned in the assay indications should be validated.
- MSI test should be tested with methods such as PCR fragment analysis or real-time PCR assays. e.g., MSI concordance to a MSI-PCR assay that detects 5 mononucleotide microsatellite loci including MR-21, BAT-25, MONO-27, NR-24 and BAT-26 can be used for MSI method comparison studies.

1) Scope

2) Background

3) Risk Class

4) Information Required

Interrogated regions

Sample processing

DNA/RNA extraction

Library construction

Sequencing & data analysis

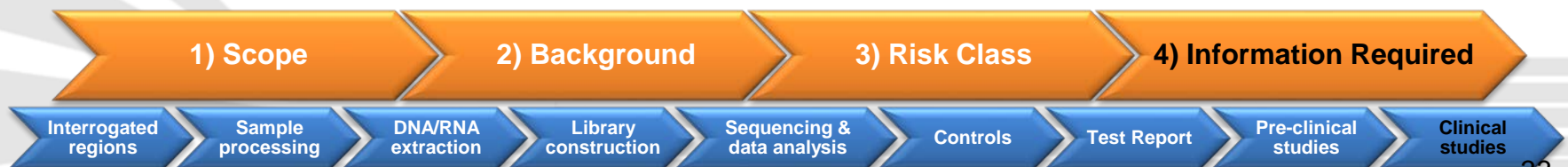
Controls

Test Report

Pre-clinical studies

Clinical studies

- Tumour mutational burden (TMB) analysis can be compared against other validated NGS assays with identical bioinformatics pipeline/threshold values used for the subject NGS IVD test kit. Concordance rate of positive percent agreement and negative percent agreement with comparison method should be provided.
- Clinical evidence curation method/literature review should be provided for the genomic variants included in the assay.



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