NYSDOH Initial Validation of the Oncomine Precision Assay for Use in Clinical Studies

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.Introduction

- The Oncomine Precision Assay with the Genexus Integrated Sequencer (OPA GX) is a pan-cancer research next-generation sequencing (NGS) panel designed for detecting cancer driver variants across 50 genes.
- Variant types include single nucleotide variants (SNV), insertions and/or deletions (INDEL), copy number variants (CNV) and gene fusions.
- The Genexus platform produces results with a quick turnaround time (in as little as one day), enabling faster patient enrollment in clinical trials.
- OPA GX utilizes Ion AmpliSeq-HD chemistry, allowing for DNA and RNA inputs as low as 13.4 ng of extracted material from formalin-fixed paraffin embedded (FFPE) tumor samples.
- Initial validation was performed taking into consideration elements of New York State Department of Health (NYSDOH) guidelines [1] in conjunction with Jennings *et al.* [2], demonstrating its effectiveness to detect somatic variants in formalin-fixed paraffin-embedded (FFPE) tumor samples.
- Initial validation included assessment of OPA GX performance characteristics including Limit-of-Detection (LOD), Orthogonal Confirmation, Analytical Accuracy and Reproducibility and Precision across a range of variants specifically for SNV and INDEL.

Materials and Methods

Sample Tested

- A combination of FFPE tumor samples and cell lines harboring known mutations were used to evaluate OPA GX performance characteristics
  - FFPE specimens, n=93
  - Cell lines, n=5

Figure 1. OPA GX Workflow from Sample to Data Analysis

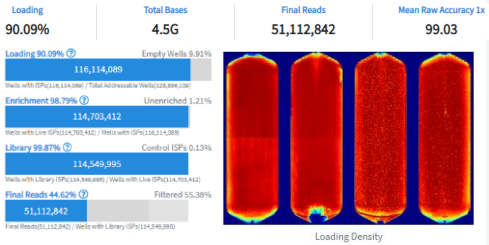
Materials and Methods (cont.)

Figure 2. GX5 Chip and Genexus Integrated Sequencer

Workflow

- Following pathology assessment and macrodissection, FFPE and cell lines underwent DNA and RNA extraction utilizing the MagMAX FFPE DNA/RNA Ultra Kit on the KingFisher Flex Purification System.
- Libraries were automatically prepared, templated and sequenced on the Genexus Integrated Sequencer utilizing 13.4 ng of nucleic acid input.

Figure 3. Representative Genexus Software Sequencing Output



Determination of Performance Characteristics

Limit of Detection (LOD) and Analytical Sensitivity:

- LOD refers to the allelic fraction where variants can be reliably determined with a certain degree of confidence, >95% sensitivity
  - Two cell lines, SNV (*KRAS*) and INDEL (*EGFR*), were diluted to five minor allele frequencies (MAF) between 10% and 2.5% with normal gDNA
  - LOD verification was carried out using 2 SNV and 2 INDEL FFPE patient samples with an average MAF of 8.2% and 8.0%, respectively.

Materials and Methods (cont.)

Table 1. LOD Performance for SNV and INDEL

	SNV (*KRAS*)		INDEL (*EGFR*)	
MAF	Replicates with Variants Detected	Sensitivity	Replicates with Variants Detected	Sensitivity
10%	20/20	100%	20/20	100%
8%	20/20	100%	20/20	100%
6%	20/20	100%	20/20	100%
4%	20/20	100%	20/20	100%
2.5%	11/20	55%	9/20	45%

Orthogonal Confirmation

- A total of 89 FFPE samples (49 SNV, 10 INDEL, and 30 wild-type) were sequenced and then orthogonally-confirmed via Sanger or Oncomine Focus Assay (OFA) sequencing.
- Overall percentage agreement across 89 FFPE samples was 100%.

Analytical Accuracy

- Three commercial reference cell lines were sequenced in quadruplicate to calculate analytical accuracy.
- Percentage agreement was calculated as follows:

$$= \frac{(TP+TN)}{(TP+TN+FP+FN)} * 100\%$$

- Overall percentage agreement across 1770 variants targeted by OPA GX was 100%

Reproducibility

- Assess assay variability for each variant type (SNV and INDEL) across multiple combinations of operators and instruments to achieve a >95% reproducibility.
  - Five samples were run in quadruplicate or quintuplicate to capture a minimum of 20 replicates per variant type.

Precision

- Evaluates the variability in variant calls within a run
  - Three positive samples for each variant type (SNV and INDEL) were run in triplicate within the same run.

Results

Table 2. OPA GX Performance Characteristic Results for SNV and INDEL

Performance Characteristic	SNV	INDEL
	> 4% MAF	> 4% MAF
Sensitivity	>99%	>99%
Orthogonal Confirmation	100%	100%
Accuracy	>99%	>99%
Reproducibility	>99%	>99%
Precision	>99%	>99%

Conclusions

- Initial validation shows targeted sequencing with the Oncomine Precision Assay panel exceeds the analytical accuracy and sensitivity, orthogonal confirmation, precision, and reproducibility performance criteria set by NYSDOH guidelines.
- All performance characteristics evaluated were at 99% or above for a wide array of biologically relevant variants
- The Genexus platform's rapid turnaround time demonstrates the suitability for the Oncomine Precision Assay to be used in clinical settings with a highly automated workflow.

References

1.Next Generation Sequencing (NGS) guidelines for somatic genetic variant detection. New York State Department of Health. 2021. https://www.wadsworth.org/sites/default/files/WebDoc/NextGenSeqONCOGuidelines%20_April_2021.pdf

2.Jennings LJ, Arcila ME, et al. Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn. 2017 May; 19(3):341-365

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