Clinical Verification of an FDA-Approved NGS Solid Tumor IVD Assay

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INTRODUCTION

- In light of the recent FDA final ruling regarding increased oversight of laboratory developed tests (LDTs), implementation of FDA-approved assays may provide an avenue for affordable compliance in clinical reference and hospital laboratories.
- The Pillar Biosciences' oncoReveal™ CDx Pan Cancer Solid Tumor IVD assay is a 22 gene amplicon-based target enrichment NGS assay with FDA approval at all steps from library preparation, sequencing, bioinformatic analysis, and qualitative reporting, including a companion diagnostic (CDx) of clinically significant single nucleotide variants (SNVs) and small insertions and deletions.
- This assay is marketed with a detection rate as low as 1.5% variant allele frequency (VAF) in SNVs, 2.2% for insertions, and 1.6% for deletions.

NCCN Guidelines Version 5.2024 Colon Cancer (CRC)

- All patients with metastatic CRC should have tumor genotyped for RAS (**KRAS** and **NRAS**) and **BRAF** mutations individually or as part of a next-generation sequencing (NGS) panel (preferred).
- Patients with any known **KRAS** mutation (exons 2, 3, and 4) or **NRAS** mutation (exons 2, 3, and 4) should not be treated with either cetuximab or panitumumab, unless given as part of a regimen targeting a **KRAS G12C** mutation.
- **BRAF V600E** mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor.

Genes and Codons Tested in oncoReveal™ CDx Pan Cancer Solid Tumor IVD Assay

Gene	Exon	Codons Covered
AKT	3	17-42
ALK	22	1151-1171
	23	1174-1206
	25	1256-1278
BRAF	11	439-472
	15	582-609
CTNNB1	3	2-46
DDR2	5	99-139
	8	229-261
	12	453-488
	13	512-547
	14	587-619
	15	624-660
	17	762-784
EGFR	3	95-128
	7	275-296
	12	457-492
	15	588-621
	18	696-726
	19	729-761
	20	762-800
	21	846-875
ERBB2	19	754-769
	20	770-805
	21	840-878
ERBB4	3	109-140
	4	166-185
	6	223-247
	7	260-288
	8	296-323
	9	334-359
	15	591-622
	23	918-948
FBXW7	5	250-287
	8	382-406
FBXW7	9	445-472
	10	479-508
	11	560-592
FGFR1	4	121-147
	7	250-285
FGFR2	7	251-279
	7	297-313
	9	369-404
	12	532-557
FGFR3	7	248-274
	9	368-401
	14	633-653
	16	678-716
	18	784-807
KRAS	2	4-37
	3	39-73
	4	113-147
MAP2K1	2	32-68
MET	2	153-185
	2	354-385
	11	826-866
	14	986-1019
	16	1108-1131
	19	1243-1277
NOTCH1	26	1569-1602
	27	1674-1679
NRAS	2	1-35
	3	42-69
	4	117-150
PIK3CA	2	82-117
	5	315-352
	7	389-417
	8	418-422
	10	538-554
	14	692-723
PIK3CA	19	890-909
	21	1016-1050
	21	1066-1069
PTEN	1	1-26
	3	56-69
	5	98-140
	5	153-164
	6	165-178
	7	213-215
	7	230-267
	8	276-301
	8	312-342
SMAD4	3	100-132
	4	143-151
	5	164-198
	6	242-262
	8	309-318
	9	330-362
	10	383-413
	11	443-473
	12	499-540
STK11	1	23-62
	4	193-199
	6	247-281
	8	323-361
TP53	2	1-24
	4	82-114
	5	118-223
	5	126-138
	6	150-186
	7	225-256
	8	263-306
	10	332-366

METHODOLOGY

- A clinical verification of the assay was performed using 20 DNA samples extracted from formalin-fixed, paraffin-embedded tissue from colon, lung, and skin.
- The MiSeq™ Dx instrument (Illumina) and oncoReveal™ CDx PiVAT® software were used for sequencing and data analysis, respectively.
- Accuracy was assessed based on concordance with results generated using a clinically-validated solid tumor NGS LDT.
- Reproducibility, sensitivity, and specificity were verified across different tumor types, assay runs, and operators, generating a total of 40 libraries.

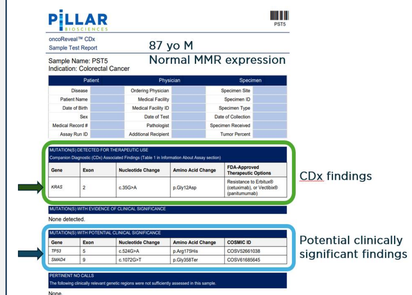
RESULTS

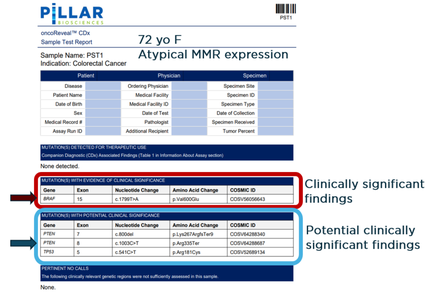
- Mutational status was concordant in all samples compared to the reference results, resulting in an overall accuracy of 100%.
- Inter- and intra-assay and inter-operator reproducibility all yielded results of 100%.
- The data showed no evidence of false positives or negatives, performing at 100% for analytical sensitivity and specificity.

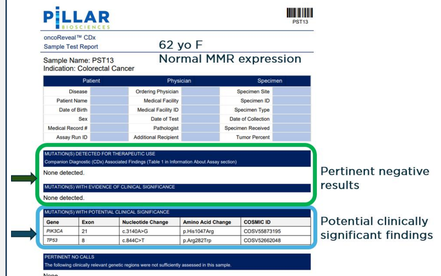
TST15 Assay		Pillar oncoReveal CDx Panel Cancer Solid Tumor		
		Positive	**Negative (WT)**	**Total**
	Positive	A (26)	B (0)	A+B (26)
	Negative (WT)	C (0)	D (134)	C+D (134)
	Total	A+C (26)	B+D (134)	A+B+C+D (160)

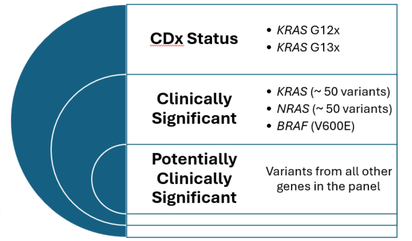
Parameter	Result
Sample Concordance	100%
Variant Call Agreement	100%
Intra-assay Reproducibility	100%
Inter-assay Reproducibility	100%
Inter-operator Reproducibility	100%
Analytical Sensitivity	100%
Analytical Specificity	100%

EXAMPLE REPORTS









CONCLUSIONS

BENEFITS

- **Robust** assay requiring ≥ 30% tumor burden and > 4.5 ng/µL of DNA.
- **Streamlined** wet lab process.
- **Scalable** library prep (6-46 samples + controls) that can be completed in one day.
- **Accessible** sequencing process that utilizes a MiSeqDx.
- **Easy-to-interpret** reports generated within a few hours on CDx server.
- **FDA-approved** assay with companion diagnostic component.
- **Reimbursable** as a small NGS panel.

LIMITATIONS

- Difficulty achieving passing criteria with limited tumor specimens.
- CDx indications limited to EGFR and KRAS.
- VAFs are not currently included in the clinical report.

ACKNOWLEDGMENTS