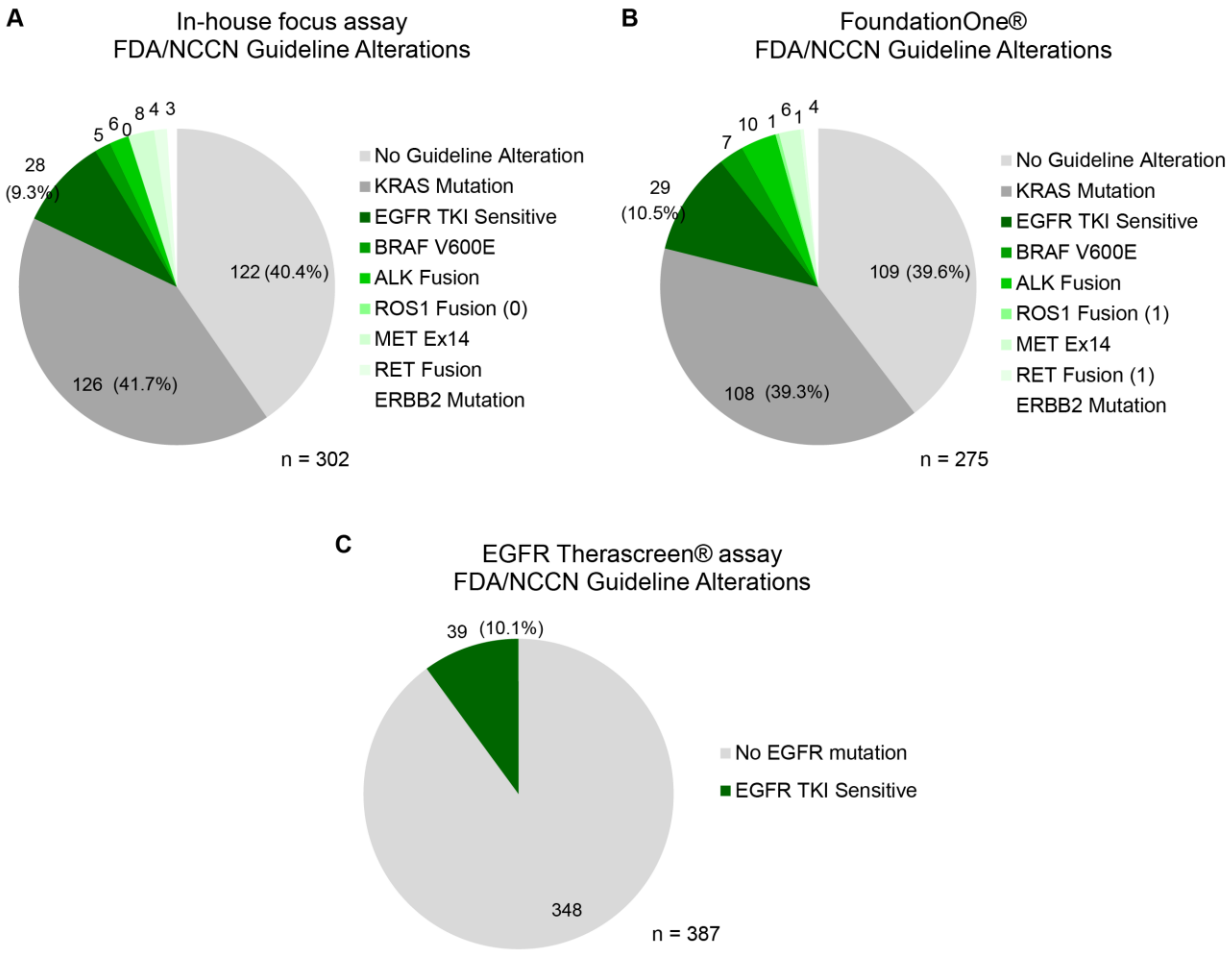
**Supplemental Data**

**Supplemental Table 1: Solid Tumor Focus Assay Gene list.** Targeted regions from 69 genes are assayed. The Oncomine™ Focus Assay was the base assay and it was expanded in-house with 17 alterations in the genes highlighted in red. This assay is designed to detect known clinically important single nucleotide variants, small insertions and deletions, high copy number amplifications, and gene rearrangements. Not all exons of all genes are assessed nor are all exons evaluated in their entirety

| Hotspots Genes | | | | Amplification (CNV) Genes | | Gene Fusions | |
|---|---|---|---|---|---|---|---|
| AKT1 | EP300 | IDH2 | PDGFRA | ALK | KIT | ABL1 | MET |
| ALK | ERBB2 | JAK1 | PIK3CA | AR | KRAS | ALK | NTRK1 |
| APC | ERBB3 | JAK2 | POLE | AXL | MET | AKT3 | NTRK2 |
| AR | ERBB4 | JAK3 | PTEN | BRAF | MYC | AXL | NTRK3 |
| ARAF | ESR1 | KEAP1 | RAC1 | CCND1 | MYCN | BRAF | PDGFRA |
| B2M | FBXW7 | KIT | RAF1 | CDK4 | PDGFRA | EGFR | PPARG |
| BRAF | FGFR1 | KRAS | RET | CDK6 | PIK3CA | ERBB2 | RAF1 |
| CDK4 | FGFR2 | MAP2K1 | ROS1 | EGFR | RICTOR | ERG | RET |
| CDKN2A | FGFR3 | MAP2K2 | SMAD4 | ERBB2 | | ETV1 | ROS1 |
| CREBBP | GNA11 | MET | SMO | FGFR1 | | ETV4 | |
| CTNNB1 | GNAQ | MTOR | TP53 | FGFR2 | | ETV | |
| DDR2 | HRAS | NFE2L2 | U2AF1 | FGFR3 | | FGFR1 | |
| EGFR | IDH1 | NRAS | | FGFR4 | | FGFR2 | |
| | | | | | | FGFR3 | |
| DNA | | | | | | RNA | |

Gene alterations in RED were added in expanded Solid Tumor Focus Assay

**Supplemental Table 2: Comparison of genetic alterations within patient population at UHCMC using different assays.** Alterations listed by rate within UHCMC cohort for **A**) In-house Focus Assay, **B**) FoundationOne®, and **C**) for in-house EGFR *Therascreen*® assay. Data for the in-house Focus Panel, which includes both the Oncomine™ Focus Assay and the expanded Solid Tumor Focus Assay, were collected from 2016-2018. FoundationOne® and EGFR *Therascreen*® data were collected from 2014-2016.



## Supplemental Data: Appendix 1

## Part 1- Oncomine Focus Assay Validation Summary

### Section I: Fusions (RNA) validation

| Test Category | Material Used |
|---|---|
| Analytical Sensitivity (LOD) | H2228 cell line (*EML4-ALK* and *ALK-PTPN3* fusions) RNA serially diluted (100% to 3%) into RNA from non-fusion cell line (H1975) |
| Accuracy and Specificity | 22 FFPE tumor samples analyzed against orthogonal test method(s) |
| | Reference material (Seraseq FFPE Fusion RNA-I) containing 10 gene fusions |
| Precision and Reproducibility | 6 H2228 cell line dilutions analyzed within and between runs |
| | 5 FFPE tumor samples analyzed within and between runs |
| | Seraseq FFPE Fusion RNA-I (10 gene fusions) analyzed within and between runs |

**Limit of Detection (LoD):** RNA from H2228 cell line (known to contain EML4-ALK and ALK-PTPN3 fusions) was serially diluted in the RNA from fusion-negative cell line (NCI-H1975) resulting in dilution ranging from 3% to 100% H2228 tumor RNA. Both EML4-ALK and ALK-PTPN3 fusions were detected in all dilutions, see Table I-1. For detection of fusions, these experiments confirm a limit of detection of 3% tumor content (nuclei) for fusion detection.

**Table I-1.** Detection of two different ALK fusions in different HCC827 dilution levels.

| % HCC228 Tumor RNA | Run | Fusion reads (normalized fusion reads) | |
|---|---|---|---|
| | | EML4(6)-ALK(20) | ALK(11)-PTPN3(3) |
| 100 | A | 6253 (.046) | 4884 (.036) |
| 50 | A | 5984 (.043) | 3438 (.025) |
| 50 | B | 8083 (.036) | 5781 (.026) |
| 50 | B | 9680 (.041) | 5973 (.026) |
| 25 | A | 9374 (.037) | 2758 (.011) |
| 25 | B | 8960 (.034) | 4035 (.015) |
| 25 | B | 9666 (.038) | 3223 (.013) |
| 12.5 | A | 3502 (.025) | 1336 (.01) |
| 12.5 | B | 5965 (.028) | 1249 (.006) |
| 12.5 | B | 3575 (.031) | 955 (.008) |
| 12.5 | C | 3884 (.024) | 2526 (.016) |
| 12.5 | C | 3120 (.022) | 2094 (.015) |
| 6 | A | 4074 (.018) | 1460 (.006) |
| 6 | C | 2580 (.015) | 1616 (.009) |
| 6 | C | 2966 (.017) | 2520 (.014) |
| 3 | C | 2230 (.011) | 1651 (.008) |
| 3 | C | 2581 (.009) | 1510 (.005) |
| 0 | A | ND | ND |

Normalized fusion reads (fusion reads/total mapped RNA reads) is depicted in parentheses for each fusion. **ND**: non detected

**Limits of Quantification and Reportable Range:** Sensitivity and replication studies using H2228 cell line demonstrate a combined analytic sensitivity of a 100% [88%-100%] in detection of both *ALK* fusions (samples containing 3% to 100% H2228 RNA). Studies on FFPE clinical tumor samples (tumor nuclei ranging for 20% to 70%) demonstrate analytic sensitivity of 100% [83%-100%] in detection of fusions (5 different common fusions tested). The clinically reportable range for fusions will be alterations that meet 'call' thresholds as determined by studies with H2228 cell line (Table I-2), which have been demonstrated to empirically hold in FFPE clinical tumor samples containing 20% to 70% tumor nuclei (expected test samples for assay), see Table I-3.

**Table I-2.** Sequencing Metrics: Minimum Criteria for Calling Fusion Positive

| Metric | Threshold | Minimum Observed | Maximum Observed |
|---|---|---|---|
| Total Mapped RNA Reads | 10,000 | 113,876 | 285,797 |
| Fusion Reads | 500 | 955 | 9680 |
| Normalized Fusion Reads (fusion reads/total mapped RNA reads) | .005 | .005 | .046 |

- The values are derived from sensitivity and reproducibility experiments with H2228 cell line.

**Accuracy**: We evaluated the accuracy of the assay in its ability to detect fusions in FFPE clinical samples and characterized reference material. Results from clinical FFPE samples were compared with expected results seen by alternative NGS methodology (Foundation One; ArcherDx FusionPlex) and FDA-approved ALK IHC and/or ALK FISH testing. The 9 confirmed detected (positive) cases are shown in Table I-3. There were 13 confirmed negative cases, zero false positive, and zero false negative cases. Given the infrequent occurrence of many of the fusion in our clinical sample set, the Seraseq FFPE Fusion RNA reference material was used to increase the number of different variants tested on the panel. All 10 key fusions were detected and pass sequencing metrics detailed above, see Table I-4.

Positive percent agreement (PPA;accuracy): Clinical + reference FFPE samples: 100% [79%-100%]
Positive predictive value (PPV): Clinical + reference FFPE samples: 100% [79%-100%]
Specificity: Clinical + reference FFPE samples: 100% [72%-100%]

**Table I-3.** Gene fusions detected in FFPE clinical samples

| Sample | Tumor % | Gene Fusion Detected | Confirmation Method | Fusion Reads | Mapped RNA Reads | Normalized Fusion Reads |
|---|---|---|---|---|---|---|
| OFA_2 | 40 | *MET*(13)-(15) (exon 14 skip alteration) | NGS(FO) | 22103 | 224723 | .098 |
| OFA_7 | 30 | *EML4*(6)-*ALK*(20) (variant 3a/b) | NGS(FO; ADx); IHC | 583 | 112647 | .005 |
| OFA_11 | 20 | *EML4*(6)-*ALK*(20) (variant 3a/b) | NGS(FO; ADx); IHC | 7487 | 151875 | .049 |
| OFA_12 | 20 | *EML4*(13)-*ALK*(20) (variant 1) | NGS(FO; ADx); IHC | 10389 | 138278 | .075 |
| OFA_13 | 70 | *EML4*(13)-*ALK*(20) (variant 1) | NGS(FO; ADx); IHC | 15602 | 181964 | .086 |
| OFA_14 | 40 | *EML4*(6)-*ALK*(20) (variant 3a/b) | NGS(FO; ADx); IHC | 8806 | 188895 | .047 |
| OFA_17 | 30 | *MET*(13)-(15) (exon 14 skip alteration) | NGS(FO) | 21444 | 181627 | .118 |
| OFA_18 | 40 | *EML4*(18)-*ALK(20)* fusion (variant 5) | NGS(FO; ADx); IHC | 45606 | 267877 | .170 |
| OFA_19 | 50 | *KIF5B(15)-RET(12)* | NGS(FO; ADx) | 47531 | 317170 | .150 |

**FO**: Foundation Medicine One Testing; **ADx**: ArcherDx Fusion Plex Testing; **IHC**: FDA-approved Ventana ALK IHC

**Table I-4.** Gene fusions detected in Seraseq FFPE Fusion RNA reference material.

| RNA Fusion | Fusion Reads | Normalized Fusion Reads | COSMIC/NCBI ID | OFA Variant ID |
|---|---|---|---|---|
| EML4(13) - ALK(20) | 10819 ± 2096 | .044 ± .001 | AB462411 | EML4-ALK.E13A20.AB462411 |
| KIF5B(24) - RET(11) | 11729 ± 2307 | .048 ± .001 | COSF1262 | KIF5B-RET.K24R11.COSF1262 |
| NCOA4(7) - RET(12) | 10311 ± 2592 | .042 ± .004 | | NCOA4-RET.N7R12 |
| CD74(6) - ROS1(34) | 4843 ± 1017 | .020 ± .002 | COSF1200 | CD74-ROS1.C6R34.COSF1200 |
| SLC34A2(4) - ROS1(34) | 5701 ± 1154 | .023 ± .002 | COSF1198 | SLC34A2-ROS1.S4R34.COSF1198 |
| TPM3(7) - NTRK1(10) | 12106 ± 2955 | .049 ± .004 | COSF1318 | TPM3-NTRK1.T7N10.COSF1318 |
| ETV6(5) - NTRK3(15) | 16151 ± 3271 | .066 ± .001 | COSF571 | ETV6-NTRK3.E5N15.COSF571.1 |
| FGFR3(17) - TACC3(11) | 12592 ± 1832 | .052 ± .006 | | FGFR3-TACC3.F17T11 |
| FGFR3(17) - BAIAP2L1(2) | 7657 ± 1671 | .031 ± .001 | COSF1347 | FGFR3-BAIAP2L1.F17B2.COSF1347 |
| PAX8(9) - PPARG(2) | 8223 ± 1725 | .034 ± .003 | COSF1217 | PAX8-PPARG.P9P2.COSF1217 |

**Precision and Reproducibility:**

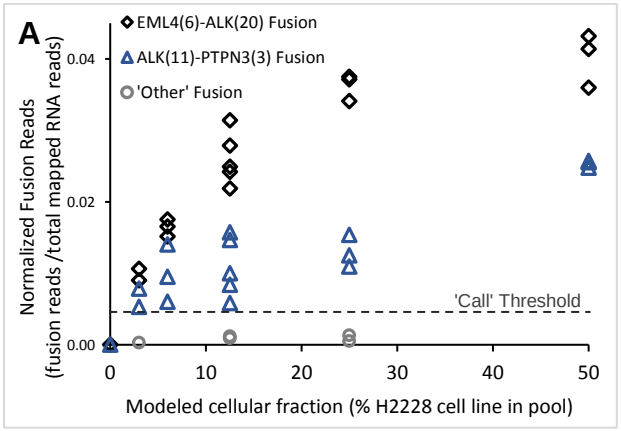
Intra-run experiments: (1) Six different H2228 cell line dilutions ranging from 3% to 50% tumor (*EML4-ALK* and *ALK-PTPN3* fusions) were run in duplicate on the same run. The two expected fusions were detected in all 6 dilutions. (2) Five different clinical FFPE tumor samples (with tumor content ranging from 20% to 60%) were each run in triplicate. The

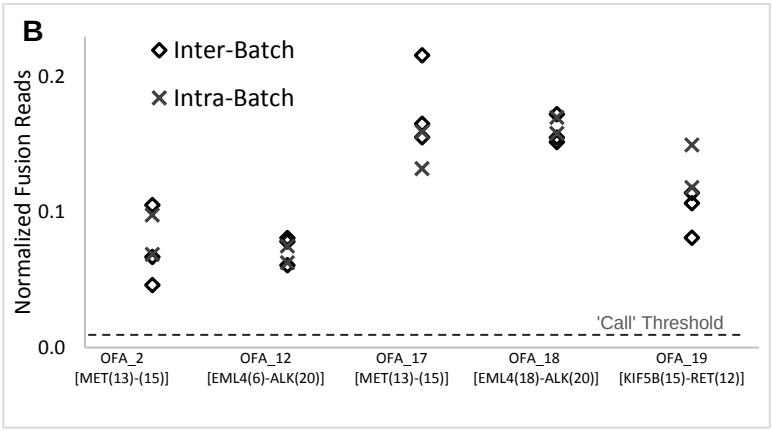
expected fusion was correctly called (detected above threshold) in all samples tested. (3) Seraseq FFPE Fusion RNA-I reference material were processed in duplicate in the same batch and run. The 10 expected fusions were correctly detected above threshold in all samples tested (mean coefficient of variation (CV) of .016% for normalized fusion reads) with 100% specificity. (4) A negative control (normal lung RNA) was run in triplicate and no fusions were detected.

Inter-run experiments: (1)Three different H2228 cell line dilutions ranging from 6% to 25% tumor were run on two different runs (6% and 25% dilution) or three different runs (12% dilution), on different days, with different reagents.. The two expected fusions were detected in all 3 dilutions. (2) Five different clinical FFPE tumor samples (with tumor content ranging from 20% to 60%) were each run on two different runs, on different days, with different reagents. The expected fusion was correctly called (detected above threshold) in all samples tested. (3) Seraseq FFPE Fusion RNA-I reference material was run on two different batches on different days. The 10 expected fusions were correctly detected above threshold in all samples tested (mean CV of 0.02% for normalized fusion reads) with 100% specificity.

Overall Intermediate Precision: 100% [90% - 100%]
Summary of precision experiments for H2228 cell line (Figure I-1A) and FFPE clinical tumor samples (Figure I-1B) are shown below.

**Figure I-1A/B. Precision studies:** Chart showing normalized fusion reads for in precision studies involving (A) H2228 dilution samples and (B) FFPE clinical NSCLC tumor samples. Each symbol represents finding in an individual sample. All 'called' samples reached 500 fusion reads threshold.




*Dilutions were run over three different runs spanning 3 months.

*Each sample was run in quadruplicate over two different runs (3 months apart).

**Results of Sequencing Metrics in FFPE Clinical Samples (RNA):**

**Table I-5.** Sequencing metrics observed based on testing in clinical FFPE samples. Thresholds established based on experiments with ALK fusion (H2228) cell line, see Figure III-2.

| Metric | Threshold | Minimum Observed | Maximum Observed | Mean | Median |
|---|---|---|---|---|---|
| Total Mapped Fusion Reads | 10,000 | 29,194 | 436,285 | 205,557 | 212,631 |
| Fusion Reads | 500 | 583 | 47531 | 24934 | 23055 |
| Normalized Fusion Reads | 0.005 | 0.005 | 0.216 | 0.107 | 0.102 |

## II. Single Nucleotide Variants (SNV) and InDel(s) (DNA) Validation

| Test Category | Material Used |
|---|---|
| Analytical Sensitivity (LOD) | Characterized reference material (quantified by ddPCR) containing 33 variants ranging from 3-5% allele frequency were tested |
| Accuracy and Specificity | 44 FFPE tumor samples analyzed against orthogonal test method(s) |
| | Characterized reference material (Horizon diagnostics, Oncology hotspot control, CAP proficiency samples) testing 200 SNVs (VAF: 3-35%) and 10 indels (VAF: 2-35%) |
| Precision and Reproducibility | Characterized reference material (QMRS, AOHC) were analyzed within and between runs |

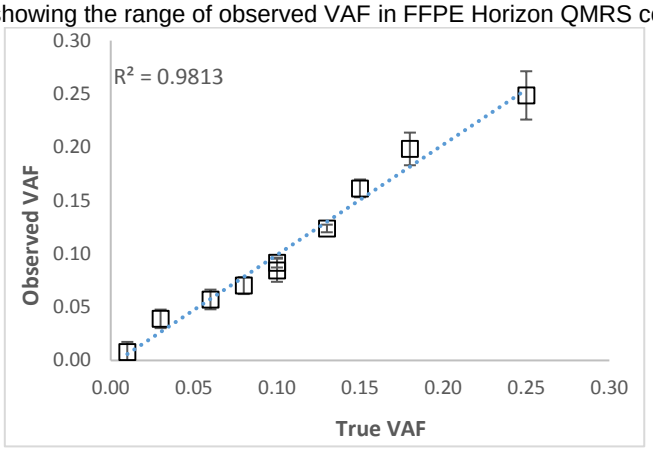
**Limit of Detection (LoD):** Reference material from Horizon Diagnostics in which the variants were quantified by qqPCR were analyzed focusing on 33 variants with VAF ranging from 3-6%. All 33 variants were detected and observed in the expected 95% CI for each variant, see Table II-1. These results demonstrate an empirical limit of detection of VAF of 3%. To be conservative, a VAF of 5% will be the stated limit of detection.

**Table II-1.** Detection of low frequency clinically-relevant variants in quantified reference material.

| Gene | Variant | Expected VAF | Observed VAF | 95% CI - Observed VAF at 500X coverage | Reference Standard |
|---|---|---|---|---|---|
| *ALK* | F1174L | 5.0% | 5.5% | 3.4% - 7.3% | Horizon Tru-Q2 |
| *BRAF* | V600G | 4.0% | 4.8% | 2.6% - 6.1% | Horizon Tru-Q2 |
| *BRAF* | V600K | 4.0% | 3.1% | 2.6% - 6.1% | Horizon Tru-Q1 |
| *BRAF* | V600M | 4.0% | 3.8% | 2.6% - 6.1% | Horizon Tru-Q3 |
| *BRAF* | V600R | 4.0% | 4.1% | 2.6% - 6.1% | Horizon Tru-Q4 |
| *EGFR* | L858R | 4.2% | 3.0% | 2.7% - 6.2% | Horizon Tru-Q2 |
| *EGFR* | L858R | 3.0% | 4.0% | 1.8% - 4.9% | Horizon QMRS |
| *EGFR* | L861Q | 4.2% | 4.3% | 2.7% - 6.2% | Horizon Tru-Q4 |
| *EGFR* | T790M | 4.2% | 3.6% | 2.7% - 6.2% | Horizon Tru-Q1 |
| *GNA11* | Q209L | 5.0% | 5.1% | 3.4% - 7.3% | Horizon Tru-Q3 |
| *GNAQ* | Q209L | 5.0% | 5.9% | 3.4% - 7.3% | Horizon Tru-Q2 |
| *IDH1* | R132C | 5.0% | 5.0% | 3.4% - 7.3% | Horizon Tru-Q1 |
| *IDH1* | R132H | 5.0% | 6.1% | 3.4% - 7.3% | Horizon Tru-Q3 |
| *IDH2* | R140Q | 5.0% | 4.5% | 3.4% - 7.3% | Horizon Tru-Q2 |
| *IDH2* | R172K | 5.0% | 4.1% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *JAK2* | V617F | 5.0% | 5.0% | 3.4% - 7.3% | Horizon Tru-Q1 |
| *KIT* | D816V | 5.0% | 4.8% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *KRAS* | G12A | 5.0% | 4.3% | 3.4% - 7.3% | Horizon Tru-Q1 |
| *KRAS* | G12C | 5.0% | 4.5% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *KRAS* | G12D | 5.0% | 4.6% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *KRAS* | G12R | 5.0% | 5.8% | 3.4% - 7.3% | Horizon Tru-Q1 |
| *KRAS* | G12S | 5.0% | 5.1% | 3.4% - 7.3% | Horizon Tru-Q3 |
| *KRAS* | G12V | 3.8% | 4.4% | 2.4% - 5.7% | Horizon Tru-Q2 |
| *KRAS* | Q61H | 5.0% | 4.9% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *KRAS* | Q61L | 5.0% | 5.7% | 3.4% - 7.3% | Horizon Tru-Q2 |
| *KRAS* | A146T | 5.0% | 3.9% | 3.4% - 7.3% | Horizon Tru-Q3 |
| *NRAS* | Q61H | 5.0% | 5.6% | 3.4% - 7.3% | Horizon Tru-Q3 |
| *NRAS* | Q61K | 5.0% | 5.1% | 3.4% - 7.3% | Horizon Tru-Q1 |
| *NRAS* | Q61L | 5.0% | 7.1% | 3.4% - 7.3% | Horizon Tru-Q2 |
| *NRAS* | Q61R | 5.0% | 5.8% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *PDGFRA* | D842V | 5.0% | 5.0% | 3.4% - 7.3% | Horizon Tru-Q4 |
| *PIK3CA* | E542K | 5.0% | 4.2% | 3.4% - 7.3% | Horizon Tru-Q3 |
| *PIK3CA* | E545K | 5.0% | 5.7% | 3.4% - 7.3% | Horizon Tru-Q2 |

**Limits of Quantification and Reportable Range:** A commercial FFPE reference control (variants quantified by ddPCR) were analyzed four times in three different runs. We measured the range of observed VAF at known common pathogenic cancer hotspots within our targeted panel and compare them to their respective expected values (ranging from 1.0% to 24.5%). A linear reportable range is observed, see Figure II-2.

**Figure II-2.** Boxplot showing the range of observed VAF in FFPE Horizon QMRS control (n=4).




**Accuracy**: (1) One of the objectives was to evaluate the accuracy of the assay in its ability to detect relevant mutations in well-characterized reference standard materials (Horizon diagnostics quantified standards: QMRS and TRU-Q1-4 5%; AcroMetrix Oncology Hotspot Control™, and pool of CAP proficiency samples). All reference material have been reported to be validated by at least one alternative method. All 200 SNVs (VAF ranging from 3-35%) and 10 indels (VAF 2-35%) covered by this panel were detected. Given that the OFA assay focuses on clinically relevant mutations, we focused our accuracy studies on well-characterized reference material that better represented these targeted regions and emphasize clinically relevant mutations. (2) The second objective was to evaluate of the accuracy of the assay in its ability to detect mutations in FFPE clinical samples which were confirmed by an alternate method. 44 FFPE samples, including 39 advanced stage NSCLC samples were evaluated by the OFA assay. The samples had orthogonal testing by FDA-approved CDx for EGFR (Therascreen, *n*=30) or validated CGP NGS (Foundation Medicine, FM; *n*=29). For complete list of samples, validation method, expected results and called results please refer to Table 2-'Validation Sample Master List' in the appendix. All 22 SNVs and 10 insertion/deletions (<30bp) were detected.

| | Reference Material | FFPE clinical samples | Reference + clinical samples |
|---|---|---|---|
| PPA (accuracy) | 100% [98%-100%] | 100% [82%-100%] | 100% [98%-100%] |
| PPV | 100% [66%-100%] | 100% [66%-100%] | 100% [80%-100%] |

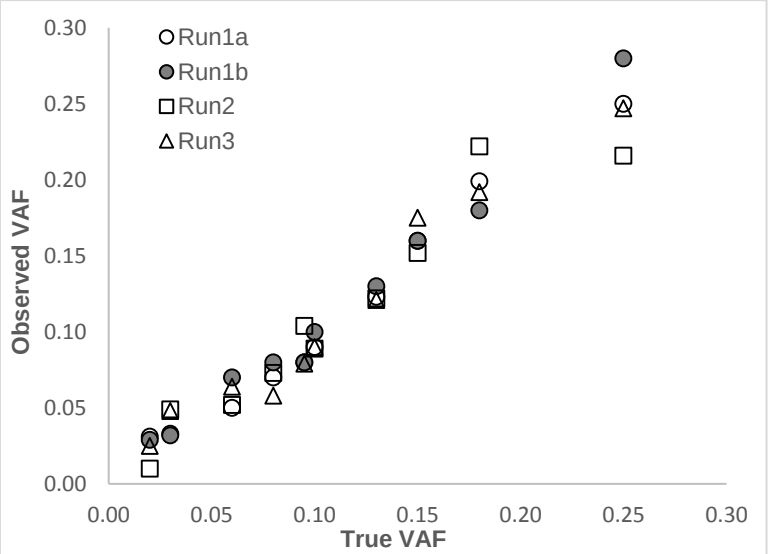
*[brackets] represent 95% confidence interval

**Precision and Reproducibility:**

Intra-run experiments: Two different characterized reference standard materials (Horizon QMRS™ and AcroMetrix Oncology Hotspot Control™) containing 140 SNVs (VAF 3-35%) and 10 indels (VAF 2-35%) were then processed in duplicate in the same batch and run. One SNV (*IDH2* c.474A>G) expected at VAF of 5% was not detected in one of the duplicate runs. All other variants were detected on both runs, with a mean coefficient of variation (CV) of 0.06% for allele frequencies.

Inter-run experiments: Horizon QMRS™ and AcroMetrix Oncology Hotspot Control™ reference materials containing 140 SNVs (VAF 3-35%) and 10 indels (VAF 2-35%) were then processed on three different batches and on three different runs. One indel (*PDGFRA* c.1698_1912del15) expected at VAF of 8% was not detected in one of the three triplicate runs. All other variants were detected on both runs, with a mean coefficient of variation (CV) of 0.1% for allele frequencies. Of note the missed indel is below what will be reported as the sensitivity of the assay for indels (VAF ≥10%).

**Figure II-4.** Plot shows observed VAF in FFPE Horizon QMRS control in reproducibility studies. (Samples labeled Run '1a' and '1b' were sequenced on the same run and batch.)




Overall Intermediate Precision:
SNVs: VAF 5-10%: 99.3% [95.8% - 99.9%]; VAF >10%: 100% [98.9% - 100%]
InDels: VAF ≥10%: 100% [80%-100%]

## III. High Copy Number Amplification (DNA) Validation

| Test Category | Material Used |
|---|---|
| Analytical Sensitivity (LOD) | DNA of 5 tumor cell lines (NCI-H69, HCC827, BT474, SNU16, and HCC1143) bearing 9 gene amplifications (≥8 copies, ploidy<4; 7 genes) were pooled with HapMap DNA (YRI population) in ratios ranging from low to high tumor content (20% to 100%), creating a total set of 56 different gene amplifications. |
| Accuracy and Specificity | |
| Accuracy and Specificity | 26 FFPE tumor samples analyzed against orthogonal test method (Foundation One) |
| Precision and Reproducibility | HCC827 (20% and 50% dilutions) and HCC1143 (20% dilution) analyzed within and between runs |

**Limit of Detection (LoD):** DNA from 5 tumor cell lines bearing 9 high copy number (≥8) gene amplifications (7 different genes) were pooled with HapMAP DNA (YRI population) in ratios creating a total set of 56 different gene amplification (25 of which from low tumor content (20-30%) samples). High performance was achieved for amplification detection at lower tumor purities (20-30%), with an overall sensitivity of 96% [78%-100%] (24/25), see Table III-1 and III-2. For high copy number amplifications (≥8), these experiments confirm a limit of detection of 30% tumor content for gene amplification detection.

**Table III-1**. Summary of copy number amplification detection performance in cell line dilutions.

| Cell Line | Ploidy* | Gene | Copy Number* | Percentage of tumor cell line in pool with amplified gene | | | |
|---|---|---|---|---|---|---|---|
| | | | | 100% | 50% | 30% | 20% |
| NCI-H69 | 1.6 | *MYCN* | 14 | Detected | Detected | Detected | Detected |
| HCC827 | 2.8 | *CDK4* | 12 | Detected | Detected | Detected | Detected |
| HCC827 | 2.8 | *EGFR* | 12 | Detected | Detected | Detected | Detected |
| HCC827 | 2.8 | *MYC* | 8 | Detected | Detected | Detected | Detected |
| BT474 | 2.7 | *ERBB2* | 14 | Detected | Detected | Detected | Detected |
| SNU16 | 3.7 | *FGFR2* | 14 | Detected | Detected | Detected | Detected |
| SNU16 | 3.7 | *MYC* | 14 | Detected | Detected | Detected | Detected |
| HCC1143 | 3.4 | *CCND1* | 12 | Detected | Detected | Detected | Detected |
| HCC1143 | 3.4 | *MYC* | 8 | Detected | Detected | Detected | Detected |

Ploidy and copy numbers from Cosmic Cell Line Project.

**Table III-2**. Summary of copy number amplification detection performance in cell line pools.

| | Percent of tumor cell line in pool containing amplified gene | | | | |
|---|---|---|---|---|---|
| Cell Line | HCC827 | | | BT474 | NCI-H69 |
| Gene (Copy Number) | *CDK4* (12x) | *EGFR* (12x) | *MYC* (8x) | *ERBB2* (14x) | *MYCN* (14x) |
| Cell Line Pool 1 | 33% -detected | 33% -detected | 33% -detected | 33% -detected | 33% -detected |
| Cell Line Pool 2 | 40% -detected | 40% -detected | 40% -detected | 20% -detected | 40% -detected |
| Cell Line Pool 3 | 40% -detected | 40% -detected | 40% -detected | 40% -detected | 20% -detected |
| Cell Line Pool 4 | 20% -detected | 20% -detected | 20%-**not called*** | 20% -detected | 60% -detected |

**Limits of Quantification and Reportable Range:** The clinically reportable range for high copy amplifications will be in samples with tumor purity ranging from 20-100% as determined by cell line sensitivity studies. These have been demonstrated to empirically hold in FFPE clinical tumor samples containing ≥30% tumor nuclei, see Table III-3.

**Accuracy and Specificity**:

**Table III-3** – Copy number amplification detection performance.

| | Tumor Fraction (20-30%) | | | | | Tumor Fraction (≥ 30%) | | | | | Confirmation Method |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total # of amplifications | False Negatives | False Positives | PPA [95% CI] | PPV [95% CI] | Total # of amplifications | False Negatives | False Positives | PPA [95% CI] | PPV [95% CI] | |
| **FFPE Clinical Tumo Samples** | 7 | 1* | 0 | 88% [47-99%] | 100% [56-100%] | 27 | 1** | 0 | 96% [80-100%] | 100% [85-100%] | NGS (Foundation One) |
| **Cell line DNA Pools/Dilutions** | 24 | 1*** | 1 | 96% [78-100%] | 96% [78-100%] | 34 | 0 | 0 | 100% [87-100%] | 100% [87-100%] | COSMIC Cancer Cell Project |

***** MET amplification was detected at calculated 3-fold gain which is below the 5-fold gain need to be called positive by Oncomine pipeline.
******KRAS amplification was detected at calculated 4.6-fold gain is below the 5-fold gain need to be called positive by Oncomine pipeline.
*******MYC amplification was detected at calculated 3.5-fold gain is below the 5-fold gain need to be called positive by Oncomine pipeline.

**Precision and Reproducibility:**
Intra-run experiments: (1) Two different cell lines (HCC827 and HCC1143) were diluted to 20% with HapMap DNA, and were then processed in duplicate in the same batch and run. The 5 expected high copy gene amplficaitons were correctly detected above threshold in all samples tested (mean CV=2.4% for calculated copy number) with 100% specificity. (2) HCC827 cell line was diluted to 50%, and were then processed in duplicate in the same batch and run. The 3 expected high copy amplifications

were correctly detected above threshold in all samples tested (mean CV=7.8% for calculated copy number) with 100% specificity.

Inter-run experiments:
(1) Two different cell lines (HCC827 and HCC1143) were diluted to 20% with HapMap DNA, and were then processed on three different batches and on 3 different runs. The 3 expected high copy amplifications were correctly were correctly detected above threshold in all samples tested (mean CV=3.8% for calculated copy number) with 100% specificity. (2) HCC827 cell line was diluted to 50%, and were then processed on two different batches and on different runs. The 3 expected high copy amplifications were correctly detected above threshold in all samples tested (mean CV=4.6% for calculated copy number) with 100% specificity.

Overall Intermediate Precision:
20-30% tumor nuclei 100% [74.6% - 100%]
>30% tumor nuclei 100% [62.8% - 100%]

## Results of Sequencing Metrics in FFPE Clinical Samples (DNA):

**Table III-4.** Sequencing metrics observed based on testing in FFPE clinical samples. Minimum and maximum range of metrics are based on confirmed detected variants. The following thresholds were used to determine which variants to include in the analysis.

| Metric | Threshold | Minimum Observed | Maximum Observed | Mean | Median |
|---|---|---|---|---|---|
| Tumor % | >20% | 20% | 70% | 43% | 40% |
| DNA Mean Depth | >300X | 304X | 3304X | 1585X | 1524X |
| DNA Uniformity | >90% | 90.6% | 98.9% | 95.7% | 95.8% |
| % Aligned Bases | >95% | 96.9% | 99.2% | 98.6% | 98.7% |
| Mutation Allele Frequency | >0.05 | 0.06 | 0.85 | 0.33 | 0.29 |
| Tumor Copy Number | >5X | 5X | 62X | 17X | 11X |

## Part 2 - Focused Solid Tumor Assay Validation Summary

| Test Category | Material Used |
|---|---|
| Analytical Sensitivity (LOD) | Characterized reference material (TruQ1 5%; quantified by ddPCR) containing 15 variants ranging from 3-5% allele frequency were tested |
| Accuracy and Specificity | 21 FFPE tumor samples analyzed against orthogonal test method(s) |
| | Characterized reference material (AcroMetrix Oncology Hotspot Control, AOHC) testing 196 SNVs (VAF ranging from 5-35%) and 8 indels (VAF 5-35%) |
| | HapMAP pooled dilution studies ((NA18511, NA18867, NA18924, NA19108, NA19147, NA19240) |
| Precision and Reproducibility | Characterized reference material (AOHC) were analyzed within and between runs for 5 replicates |

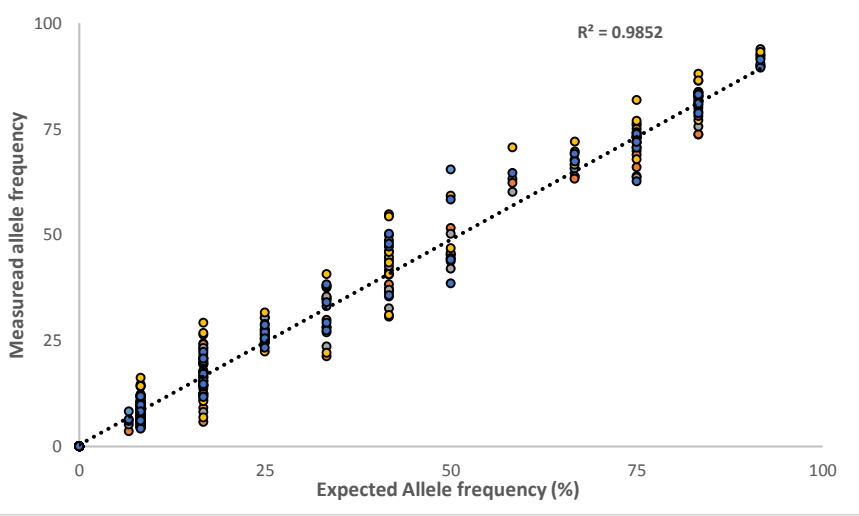
**Limit of Detection (LoD):** Reference material from Horizon Diagnostics (TruQ1 5%) in which the variants were quantified by qqPCR were analyzed. All 15 variants were detected and observed in the expected 95% CI for each variant. These results demonstrate an empirical limit of detection of VAF of 5%.

**Accuracy**:
(1) One of the objectives was to evaluate the accuracy of the assay in its ability to detect relevant mutations in well-characterized reference standard material (AcroMetrix Oncology Hotspot Control™). All reference material have been reported to be validated by at least one alternative method. All 196 SNVs (VAF ranging from 5-35%) and 8 indels (VAF 5-35%) covered by this panel were detected. This analysis was repeated five times (triplicate in one run) and two other replicates on different run each to look at inter-run and intra-run reproducibility. There was good concordance as only 2 out of possible 1020 variants were missed (99.8% accuracy) with mean coefficient of variation (CV) of 9% for allele frequencies.

(2) The second objective was to evaluate of the accuracy of the assay in its ability to detect mutations in FFPE clinical samples which were confirmed by an alternate method. 21 FFPE samples were evaluated. All 28 SNVs and 13/16 CNVs were detected. Three lower level amplification (MYC, KRAS, CCND1, <7x) by OFA assay in one sample that did not meet the threshold to be called (4.5-5.5x). Given the low tumor percentage (20%) for the sample and that therapeutic significance, which this assay designed to answer, is based on very high copy number changes, this variance between assays is acceptable. For high copy number amplifications (≥8), these experiments confirm a limit of detection of 30% tumor content for gene amplification detection.

**HapMAP SNP analysis:** HapMAP samples (NA18511, NA18867, NA18924, NA19108, NA19147, NA19240) were pooled and sequenced five times on two different runs. The expected variant frequency in each HapMAP was determined by using HapMap3 database. The expected MAF for each test base substitution in pooled samples was calculated based on the number of alternate alleles present in mix constituents and on mixing ratios. There was great concordance between expected and detected variant frequencies, see Figure I-1.

**Figure I-1.** Allele frequencies measured in pooled samples (*y* axis) match the frequencies expected based on the mixing ratios of characterized HapMap samples.



**Appendix** - **Table 2A**. Validation Sample Master List (FFPE Clinical Samples)

| SampleID | Format | Tumor % | Tissue Type | Tissue | Tumor Type | Tumor Subtype | Expected Results | Expected Results Methodology | Applicable Validation Element | Concordant | Replicates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OFA_1 | FFPE | 40 | Surgical | Ovary | Breast | Carcinoma NOS | *MYC* amp, *CCND1* amp | NGS (FM) | Accuracy | Yes | |
| OFA_2 | FFPE | 40 | Surgical | Lung | NSCLC | Lung Carcinoma NOS | *MET* exon 14 alt splice (3028_3208+1GG>TT) | NGS (FM) | Accuracy; Fusion: Precision; RNA stability | Yes | **4** |
| OFA_3 | FFPE | 60 | Surgical, bx | Liver | Breast | Carcinoma NOS | *MYC* amp, *CCND1* amp, *FGFR1* amp, *ESR1 Y537S* | NGS (FM) | Accuracy | Yes | |
| OFA_4 | FFPE | 50 | Surgical, bx | Lung | Breast | Carcinoma NOS | *MYC* amp | NGS (FM) | Accuracy | Yes | |
| OFA_5 | FFPE | 60 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *MYC* amp | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes | |
| OFA_6 | FFPE | 40 | Surgical, bx | Liver | Breast | Carcinoma NOS | *PIK3CA* H1047R | NGS (FM) | Accuracy | Yes | |
| OFA_7 | FFPE | 30 | Surgical, bx | Lymph Node | NSCLC | Lung Carcinoma NOS | *EML4-ALK* fusion (Variant 3a/b), *CDK4* amp | Ventana ALK IHC (FDA-approved CDX); NGS (FM)/(ArcherDx) | Accuracy | Yes | |
| OFA_8 | FFPE | 30 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* E709A, G719A; *MET* amp | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes* | |
| OFA_9 | FFPE | 60 | Surgical, bx | Liver | NSCLC | Non-Small Cell Carcinoma | *EGFR* amp; *MET* amp | Therascreen (EGFR CDx); NGS (FM); ALK FISH (CDx) | Accuracy | Yes | |
| OFA_10 | FFPE | 30 | Surgical, bx | Lung | NSCLC | Non-Small Cell Carcinoma | *MET* amp, *PIK3CA* R1023Q | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes | |
| OFA_11 | FFPE | 20 | Surgical, bx | Lymph Node | NSCLC | Non-Small Cell Carcinoma | *EML4-ALK* fusion (Variant 3a/b), *CDK4* amp | Ventana ALK IHC (FDA-approved CDX); NGS (FM)/(ArcherDx) | Accuracy; Fusion: Precision; RNA stability | Yes | |
| OFA_12 | FFPE | 20 | Surgical, bx | Pericardium | NSCLC | Lung Adenocarcinoma | EML4-ALK fusion (Variant 1), *MYC* amp | Ventana ALK IHC (FDA-approved CDX); NGS (FM)/(ArcherDx) | Accuracy | Yes | **4** |
| OFA_13 | FFPE | 70 | Cytology, Fluid | Pericardial Fluid | NSCLC | Lung Adenocarcinoma | EML4-ALK fusion (Variant 1), *MYC* amp | Ventana ALK IHC (FDA-approved CDX); NGS (FM)/(ArcherDx) | Accuracy | Yes | |
| OFA_14 | FFPE | 40 | Cytology, Fluid | Pericardial Fluid | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion (Variant 3a/b) | Ventana ALK IHC (FDA-approved CDX); NGS (FM)/(ArcherDx) | Accuracy | Yes | |
| OFA_15 | FFPE | 40 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *MET* amp; *FGFR1* amp; MYC amp (equ) | NGS (FM) | Accuracy | Yes | |
| OFA_16 | FFPE | 40 | Cytology, FNA | Lymph Node | NSCLC | Lung Adenocarcinoma | *EGFR* A289V, L858R; *MET* amp, *MYC* amp | NGS (FM) | Accuracy | Yes | |
| OFA_17 | FFPE | 30 | Cytology, Fluid | Pericardial Fluid | NSCLC | Lung Adenocarcinoma | *MET* exon 14 alt splice | Therascreen (EGFR CDx); NGS (FM) | Accuracy; Fusion: Precision; RNA stability | Yes | **4** |
| OFA_18 | FFPE | 40 | Surgical, bx | Lymph Node | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion (Variant 5) | Ventana ALK IHC (FDA-approved CDX); NGS (FM)/(ArcherDx) | Accuracy; Fusion: Precision; RNA stability | Yes | **4** |
| OFA_19 | FFPE | 50 | Surgical | Pleura | NSCLC | Lung Adenocarcinoma | *KIF5B-RET* fusion, *MYC* amp | Therascreen (EGFR CDx); NGS (FM)/(ArcherDx) | Accuracy; Fusion: Precision; RNA stability | Yes | **4** |
| OFA_20 | FFPE | 60 | Surgical, bx | Trachea | NSCLC | Lung Carcinoma NOS | *MET* amp | Therascreen (EGFR CDx); NGS (FM)/(ArcherDx) | Accuracy | Yes | |
| OFA_21 | FFPE | 40 | Surgical | Heart | NSCLC | Lung Adenocarcinoma | *BRAF* G466V; *CCND1* amp, *CDK4* amp, *KRAS* amp | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes | |
| OFA_22 | FFPE | 20 | Surgical | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* A767_V769dupASV | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes | |
| OFA_23 | FFPE | 30 | Surgical | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* G719C, S768I; *PIK3CA* H1047L | Therascreen (EGFR CDx); NGS (FM)/(Ampliseq) | Accuracy | Yes | |
| OFA_24 | FFPE | 50 | Surgical | Lung | NSCLC | Lung Adenocarcinoma | *BRAF* G466V; *CCND1* amp, *CDK4* amp, *KRAS* amp | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes | |
| OFA_25 | FFPE | 40 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *KRAS* G12A, amp; *MYC* amp | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes* | |
| OFA_26 | FFPE | 60 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* L858R | Therascreen (EGFR CDx) | Accuracy | Yes | |
| OFA_27 | FFPE | 30 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *FGFR1* amp; *CCDN1* amp; *MYC* amp | Therascreen (EGFR CDx), NGS (FM) | Accuracy | Yes | |
| OFA_28 | FFPE | 50 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *CCDN1* amp; KRAS G12C | Therascreen (EGFR CDx), NGS (FM) | Accuracy | Yes | |
| OFA_29 | FFPE | 50 | Surgical | Lung | NSCLC | Lung Adenocarcinoma | *KRAS G12D* | Therascreen (EGFR CDx); ALK FISH (ALK CDx) | Accuracy | Yes | |
| OFA_30 | FFPE | 60 | Surgical, bx | Lung | Breast | Carcinoma NOS | *ERBB2* amp; *CCDN1* amp | ERBB2 FISH; NGS (FM) | Accuracy | Yes | |
| OFA_31 | FFPE | 50 | Surgical, bx | Lymph Node | NSCLC | Lung Adenocarcinoma | *EGFR* E746_A750del, T790M | Therascreen (EGFR CDx); NGS (Ampliseq) | Accuracy | Yes | |
| OFA_32 | FFPE | 50 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* exon 19 deletion (EGFR E746_A750del) | Therascreen (EGFR CDx) | Accuracy | Yes | |
| OFA_33 | FFPE | 70 | Cytology, Fluid | Pleural fluid | NSCLC | Lung Adenocarcinoma | *EGFR* exon 19 deletion (EGFR E746_A750del) | Therascreen (EGFR CDx); ALK FISH (CDx); NGS (Ampliseq) | Accuracy | Yes | |
| OFA_34 | FFPE | 60 | Surgical, bx | Lymph Node | NSCLC | Lung Adenocarcinoma | *EGFR* exon 19 deletion | Therascreen (EGFR CDx); ALK FISH (CDx); NGS (Ampliseq) | Accuracy | Yes | |
| OFA_35 | FFPE | 60 | Surgical | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* exon 19 deletion | Therascreen (EGFR CDx); ALK FISH (CDx) | Accuracy | Yes | |
| OFA_36 | FFPE | 30 | Surgical, bx | Sacrum | NSCLC | Lung Adenocarcinoma | *EGFR* exon 19 deletion (EGFR E746_A750del) | Therascreen (EGFR CDx); NGS (FM) | Accuracy | Yes | |
| OFA_37 | FFPE | 20 | Cytology, FNA | Lymph Node | NSCLC | Lung Adenocarcinoma | negative | Therascreen (EGFR CDx) | Accuracy | Yes | |
| OFA_38 | FFPE | 40 | Surgical, bx | Lung | NSCLC | Lung Adenocarcinoma | *EGFR* L858R | Therascreen (EGFR CDx) | Accuracy | Yes | |
| OFA_39 | FFPE | 30 | Surgical, bx | Brain | NSCLC | Lung Adenocarcinoma | *EGFR* L858R | Therascreen (EGFR CDx); ALK FISH (ALK CDx) | Accuracy | Yes | |
| OFA_40 | FFPE | 40 | Surgical, bx | Lymph Node | NSCLC | Lung Adenocarcinoma | *EGFR* exon 19 deletion | Therascreen (EGFR CDx); ALK FISH (ALK CDx) | Accuracy | Yes | |
| OFA_41 | FFPE | 50 | Cytology, FNA | Lymph Node | NSCLC | Lung Adenocarcinoma | negative | Therascreen (EGFR CDx) | Accuracy | Yes | |
| OFA_42 | FFPE | 40 | Surgical, bx | Pericardial Tissue | NSCLC | Lung Adenocarcinoma | negative | Therascreen (EGFR CDx); ALK FISH (ALK CDx) | Accuracy | Yes | |
| OFA_43 | FFPE | 20 | Surgical, bx | Chest Wall | NSCLC | Lung Adenocarcinoma | *EGFR* E746_A750del | Therascreen (EGFR CDx) | Accuracy | Yes | |
| OFA_44 | FFPE | 40 | Surgical, bx | Lymph Node | NSCLC | Lung Adenocarcinoma | *KRAS* G12C | Therascreen (EGFR CDx); ALK FISH (ALK CDx) | Accuracy | Yes | |

**Appendix** - **Table 2B**. Validation Sample Master List (Cell Lines)

| SampleID | Format | Cell Line(s) | Tumor Type | Tumor Subtype | Expected Results | Expected Results Methodology | Applicable Validation Element | Concordant | Replicates |
|---|---|---|---|---|---|---|---|---|---|
| HCC827_100 | DNA | HCC827 cell line | NSCLC | Lung Adenocarcinoma | *EGFR* amp, *CDK4 amp* , MYC amp, *EGFR* 746_750del (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy | Yes | 1 |
| HCC827_50 | DNA | HCC827/HapMap (50:50) | NSCLC | Lung Adenocarcinoma | *EGFR* amp, *CDK4 amp* , MYC amp, *EGFR* 746_750del (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD, Precision | Yes | 3 |
| HCC827_30 | DNA | HCC827/HapMap (30:70) | NSCLC | Lung Adenocarcinoma | *EGFR* amp, *CDK4 amp* , MYC amp, *EGFR* 746_750del (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| HCC827_20 | DNA | HCC827/HapMap (20:80) | NSCLC | Lung Adenocarcinoma | *EGFR* amp, *CDK4 amp* , MYC amp, *EGFR* 746_750del (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD, Precision | Yes | 4 |
| BT474_100 | DNA | BT-474 cell line | Breast Carcinoma | Breast Carcinoma | *ERBB2* amp, PIK3CA K111N (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy | Yes | 1 |
| BT474_50 | DNA | BT-474/HapMap (50:50) | Breast Carcinoma | Breast Carcinoma | *ERBB2* amp, PIK3CA K111N (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| BT474_30 | DNA | BT-474/HapMap (30:70) | Breast Carcinoma | Breast Carcinoma | *ERBB2* amp, PIK3CA K111N (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| BT474_20 | DNA | BT-474/HapMap (20:80) | Breast Carcinoma | Breast Carcinoma | *ERBB2* amp, *PIK3CA* K111N (VAF 5-10%) | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| HCC1143_100 | DNA | HCC1143 cell line | Breast Carcinoma | Breast Carcinoma | *CCND1 amp; MYC amp* | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy | Yes | 1 |
| HCC1143_50 | DNA | HCC1143/HapMap (50:50) | Breast Carcinoma | Breast Carcinoma | *CCND1 amp; MYC amp* | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| HCC1143_30 | DNA | HCC1143/HapMap (30:70) | Breast Carcinoma | Breast Carcinoma | *CCND1 amp; MYC amp* | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| HCC1143_20 | DNA | HCC1143/HapMap (20:80) | Breast Carcinoma | Breast Carcinoma | *CCND1 amp; MYC amp* | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD, Precision | Yes | 3 |
| H69_100 | DNA | HTB-119D(NCI-H69) cell line | NSCLC | Lung Adenocarcinoma | *MYCN* amp, *PIK3CA* 106_108del (VAF >20%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy | | 1 |
| H69_50 | DNA | HTB-119D(NCI-H69)/HapMap (50:50) | NSCLC | Lung Adenocarcinoma | *MYCN* amp, *PIK3CA* 106_108del (VAF >20%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| H69_30 | DNA | HTB-119D(NCI-H69)/HapMap (30:70) | NSCLC | Lung Adenocarcinoma | *MYCN* amp, *PIK3CA* 106_108del (VAF <20%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| H69_20 | DNA | HTB-119D(NCI-H69)/HapMap (20:80) | NSCLC | Lung Adenocarcinoma | *MYCN* amp, *PIK3CA* 106_108del (VAF <20%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| SNU16_100 | DNA | CRL-5974D (SNU-16) cell line | Stomach Carcicoma | Stomach Carcicoma | *FGFR2* amp; *MYC* amp | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy | Yes | 1 |
| SNU16_50 | DNA | CRL-5974D (SNU-16)/HapMap (50:50) | Stomach Carcicoma | Stomach Carcicoma | *FGFR2* amp; *MYC* amp | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| SNU16_30 | DNA | CRL-5974D (SNU-16)/HapMap (30:70) | Stomach Carcicoma | Stomach Carcicoma | *FGFR2* amp; *MYC* amp | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| SNU16_20 | DNA | CRL-5974D (SNU-16)/HapMap (20:80) | Stomach Carcicoma | Stomach Carcicoma | *FGFR2* amp; *MYC* amp | Cell Lines (Cosmic Cell Line Project) | CNV: Accuracy, LoD | Yes | 1 |
| CL Pool 1 | DNA | HCC827/NCI-H69/BT-474 (1:1:1) | Cell Line Pool | Cell Line Pool | *MYCN* amp; ERBB2 amp; CDK4 amp; MYC amp; PIK3CA K111N (VAF >10%); EGFR amp, p.746_750del (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| CL Pool 2 | DNA | HCC827/NCI-H69/BT-474 (2:2:1) | Cell Line Pool | Cell Line Pool | *MYCN* amp; ERBB2 amp; CDK4 amp; MYC amp; PIK3CA K111N (VAF 5-10%), 106_108del (VAF <20%); EGFR amp, 746_750del (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| CL Pool 3 | DNA | HCC827/NCI-H69/BT-474 (2:1:2) | Cell Line Pool | Cell Line Pool | *MYCN* amp; ERBB2 amp; CDK4 amp; MYC amp; EGFR p.746_750del (VAF >20%), amp; PIK3CA K111N (VAF >10%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| CL Pool 4 | DNA | HCC827/NCI-H69/BT-474 (1:3:1) | Cell Line Pool | Cell Line Pool | *MYCN* amp; ERBB2 amp; CDK4 amp; MYC amp; PIK3CA K111N (VAF 5-10%), 106_108del (VAF >20%); EGFR amp, p.746_750del (VAF >20%) | Cell Lines (Cosmic Cell Line Project) | CNV, InDel: Accuracy, LoD | Yes | 1 |
| H1975 D-1 | DNA | H1975/H2228 cell lines (100:0) | NSCLC | Lung Adenocarcinoma | *EGFR* L858R, T790M (VAFs >10%); *MYC* amp (12x) | Cell Lines (Cosmic Cell Line Project) | SNV: LoD | Yes | 1 |
| H1975 D-2 | DNA | H1975/H2228 cell lines (50:50) | NSCLC | Lung Adenocarcinoma | *EGFR* L858R, T790M (VAFs >10%); *MYC* amp (6x) | Cell Lines (Cosmic Cell Line Project) | SNV: LoD | Yes | 1 |
| H1975 D-3 | DNA | H1975/H2228 cell lines (25:75) | NSCLC | Lung Adenocarcinoma | *EGFR* L858R, T790M (VAFs 5-10%); *MYC* amp (4x) | Cell Lines (Cosmic Cell Line Project) | SNV: LoD | Yes | 1 |
| H1975 D-4 | DNA | H1975/H2228 cell lines (12:88) | NSCLC | Lung Adenocarcinoma | *EGFR* L858R, T790M (VAFs <5%); *MYC* amp (2x) | Cell Lines (Cosmic Cell Line Project) | SNV: LoD | No* (varaints below threshold) | 2 |
| H1975 D-7 | DNA | H1975/H2228 cell lines (0:100) | NSCLC | Lung Adenocarcinoma | negative | Cell Lines (Cosmic Cell Line Project) | SNV: LoD | Yes | 1 |
| RNA neg cntrl | RNA | Negative Control | NA | Normal Lung Tissue | negative | NGS (ArcherDx Lugn Panel) | Fusion Specificity | Yes | 3 |
| H2228 R_100 | RNA | H2228/H1975 cell lines (100:0) | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion Variant 3a/b, *ALK-PTPN3* fusion | Cell Lines (Cosmic Cell Line Project) | Fusion: Accuracy | Yes | 1 |
| H2228 R_50 | RNA | H2228/H1975 cell lines (50:50) | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion Variant 3a/b, *ALK-PTPN3* fusion | Cell Lines (Cosmic Cell Line Project) | Fusion: LoD, Precision | Yes | 3 |
| H2228 R_25 | RNA | H2228/H1975 cell lines (25:75) | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion Variant 3a/b, *ALK-PTPN3* fusion | Cell Lines (Cosmic Cell Line Project) | Fusion: LoD, Precision | Yes | 3 |
| H2228 R_12 | RNA | H2228/H1975 cell lines (12:88) | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion Variant 3a/b, *ALK-PTPN3* fusion | Cell Lines (Cosmic Cell Line Project) | Fusion: LoD, Precision | Yes | 5 |
| H2228 R_6 | RNA | H2228/H1975 cell lines (6:94) | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion Variant 3a/b, *ALK-PTPN3* fusion | Cell Lines (Cosmic Cell Line Project) | Fusion: LoD, Precision | Yes | 3 |
| H2228 R_3 | RNA | H2228/H1975 cell lines (3:97) | NSCLC | Lung Adenocarcinoma | *EML4-ALK* fusion Variant 3a/b, *ALK-PTPN3* fusion | Cell Lines (Cosmic Cell Line Project) | Fusion: LoD, Precision | Yes | 2 |
| H2228 R_0 | RNA | H2228/H1975 cell lines (0:100) | NSCLC | Lung Adenocarcinoma | negative | Cell Lines (Cosmic Cell Line Project) | Fusion Specificity | Yes | 1 |

**Appendix** - **Table 2C**. Validation Sample Master List (Standard Reference Material)

| SampleID | Format | Reference Standard | Vendor | Expected Results | Expected Results Methodology | Applicable Validation Element | Concordant | Replicates |
|---|---|---|---|---|---|---|---|---|
| QMRS_1 | FFPE | Quantitative Multiplex Reference Standard | HDx (HD200) | 6 SNVs (VAF 2-10%) and 4 SNVs (VAF>10%) | Reference material (confirmed by ddPCR) | SNV/InDel: Accuracy; precision | Yes | 4 |
| CAP Pool 1_1 | DNA | Pool of 5 CAP proficiency samples | CAP (MTP-4,5,6; NGSST01,03) | 10 SNVs (VAF 5-10%) and 4 SNVs (VAF>10%) | Reference material (confirmed by NGS Ampliseq) | SNV/InDel: Accuracy; precision | Yes | 3 |
| CAP Pool2_1a | DNA | Pool of 2 CAP proficiency samples | CAP (MTP-6; NGSST01) | 7 SNVs (VAF>10%) | Reference material (confirmed by NGS Ampliseq) | SNV/InDel: Accuracy; precision | Yes | 2 |
| Tru-Q1 | DNA | Tru-Q1 (5% Tier)-Reference Standard | HDx (catalog# HD728) | 7 SNVs (VAF 4-8%) and 3 SNVs (VAF>10%) | Reference material (confirmed by ddPCR) | SNV/InDel: Accuracy/sensitivity | Yes | 1 |
| Tru-Q2 | DNA | Tru-Q2 (5% Tier)-Reference Standard | HDx (catalog# HD729) | 10 SNVs (VAF 4-8%) and 3 SNVs (VAF>10%) | Reference material (confirmed by ddPCR) | SNV/InDel: Accuracy/sensitivity | Yes | 1 |
| Tru-Q3 | DNA | Tru-Q3 (5% Tier)-Reference Standard | HDx (catalog# HD730) | 9 SNVs (VAF 4-8%) and 3 SNVs (VAF>10%) | Reference material (confirmed by ddPCR) | SNV/InDel: Accuracy/sensitivity | Yes | 1 |
| Tru-Q4 | DNA | Tru-Q4 (5% Tier)-Reference Standard | HDx (catalog# HD731) | 10 SNVs (VAF 4-8%) and 3 SNVs (VAF>10%) | Reference material (confirmed by ddPCR) | SNV/InDel: Accuracy/sensitivity | Yes | 1 |
| AOHC_1 | DNA | AcroMetrix Oncology Hotspot Control | ThermoFisher (catalog #969056) | 130 SNVs and 10 Indels (VAF 5-35%) | Reference material (confirmed by sanger) | SNV/InDel: Accuracy; precision | Yes | 4 |
| Seracare RNA_1 | FFPE | FFPE Tumor Fusion RNA Reference Material v1 | Seracare | 10 different fusions | Reference material | Fusion: Accuracy; precision | Yes | 3 |