NGS Implementation in a Clinical Laboratory

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Molecular Diagnostics
Sentara Healthcare
Overview

• Background
• Rational
• Test Menu Development
• Business Case
  • Alternate funding source
• NGS Utilization
  • Cystic Fibrosis (CF)
  • Cancer Hotspot v2 (CHPV2)
  • Oncomine Focus Assay (OFA)
  • Oncomine BRCA 1/2 Research Assay
  • Oncomine Myeloid Research Assay
Sentara Network

- 12 Hospital System
- >200 Physician Offices
- Own a private payer insurance
- Reference Lab is located in the flagship hospital
Sentara Reference Laboratory

Annual Test Volume

9,000,000 Tests

200,000 Molecular Tests

1500 Molecular Oncology Tests
Molecular Test Menu

Molecular Oncology
- Oncomine Focus Assay (NGS)
- EGFR
- KRAS
- BRAF
- NRAS
- JAK2

Molecular Genetics
- CFTR
- Fragile X
- SMN1
- FVL, PT, MTHFR

Molecular Infectious Disease
- HIV (viral load & genotype)
- HCV (viral load & genotype)
- HBV
- CMV
- BK
- HSV-1/-2
- BV
- Yeast
- RPP
- Bordetella
How we decide to insource a test?

- Turnaround times sensitive?
- High enough volume?
  - Review Reference Lab Utilization
  - Top 20 tests by volume or spend
- Assay available on current instruments?
- Does my staff already have competency on a similar test?
- Can I perform an equivalent test for a lower cost?
In-house testing efficiencies:

• Expense avoidance
  • Most molecular tests cost hundreds of dollars to send to reference labs for testing
  • Buy vs own analysis

• Improved TAT
  • Many molecular tests take weeks to result from reference labs
  • We perform esoteric testing weekly

• Local physician input into test menu
  • Increased communication between providers and the laboratory allows us to develop our test menu in concert with physician ordering patterns.
Next-Generation Sequencing

• Considerations
  • Cost of in-house NGS vs single gene assays and send-out testing
  • Throughput vs single gene assays
  • Provider needs
  • Guideline changes both current and future
**NGS**
- Broad
- High Throughput
- Highly multiplexed
- Expensive, but low cost per gene
- DATA
- Bioinformatics experience needed
- Long workflow, although shorter than serially testing genes
- Analytical and clinical interpretation required

**PCR-based assays**
- Very targeted
- Quick
- Inexpensive
- Less experience required
- Ideal for single gene hotspot analysis
- Data interpretation is clear
Advanced or metastatic Adenocarcinoma NSCLC

Testing should be conducted as part of broad molecular profiling.

- EGFR
- ALK
- ROS1
- BRAF
- PD-L1
NGS Efficiencies

Cost Comparison

Time Comparison

1 Gene 3 Genes 50 Genes

PCR NGS
We decided insourcing NGS was the right thing to do for our health system.
Choosing the Right Platform

- Vendor selection criteria
  - Accuracy
  - Throughput
  - Ease of workflow
  - Test menu alignment with our needs
  - Cost per sample
  - Cost of instrument
  - Reporting capabilities
  - Support after the sale
    - Instrument service
    - Bioinformatics
- Ultimately the Ion S5/Ion Chef workflow was the best fit for our organization.
Funding the Project

• We typically have one capital funding source for all laboratory equipment for our health system.
• We have an alternate funding source (strategic capital) outside of the laboratory funding source if the project meets certain criteria.
  • A minimum dollar amount
  • Must be cutting-edge and give our health system a strategic advantage
  • Has to be presented to the board for approval
• We created a project to increase the sequencing capabilities of our laboratory (NGS & Sanger sequencing) to meet the thresholds for strategic capital.
Test Menu Pipeline

Sanger Sequencing/Fragment Analysis

Prenatal Screening (Fragile X)

Cystic Fibrosis Expanded Panel

Cancer Hotspot Panel v2

Prenatal Screening (Spinal Muscular Atrophy)

Oncomine Focus Assay (OFA)

Oncomine BRCA 1/2

Hem-path (JAK2 Exon 12/13)

Oncomine Myeloid Research Assay

Next-Generation Sequencing
Business Case

• The business case showed that it was favorable to insource this testing versus paying to send-out to a our reference laboratory (37.6% internal rate of return).
  • Cost per reportable (tech time, repeat rate, control cost, validation cost, QA cost)
  • Instrument Purchases (w/depreciation)
  • Instrument maintenance
  • Construction needed for instrument
  • Did not consider lease, electrical, etc.
• The favorable business case made it easy for us to get board approval for the project.
Automated NGS Workflow

Library Prep
15 minutes hands-on
7 hours walk-away
8 samples

Templatting
15 minutes hands-on
10 hours walk-away
24-32 samples

Sequencing
15 minutes hands-on
3 hours walk-away
24-32 samples

Analysis
1.5 hours hands-on time
2.5 hours walk-away
24-32 samples

Day 1  Overnight  Day 2  Day 2
NGS Testing

- Cystic Fibrosis Carrier Screening
  - Chosen first because:
    - High volume (30-40 per week)
    - Single gene with SNPs and Indels (least complex)
    - Needed a larger panel to match our clinicians ordering patterns.
  - CF assay design was completely customized using information on CFTR from CFTR2.org.
  - Use Ion Reporter for variant calling
  - Validation was complete in 3 months using our previously tested patients from Luminex and Coriell specimens.
  - All samples correlated well.
  - Based on the validation we confirm poly-T calls by Luminex in R117H positive patients.
Validations

- Cancer Hotspot Panel v2 (CHPv2)
  - Still only SNPs and Indels, 50 genes
  - Took more time optimizing the bioinformatics piece of the assay due to the somatic nature of the mutations (need better sensitivity than germline mutations).
  - Also had to chose a vendor for reporting.
    - Variant reporting, clinical trials, treatment/resistance information
Validations

• Oncomine Focus Assay (OFA)
• More Comprehensive
  • In addition to SNPs, MNVs, and INDEL mutations we had to validate RNA fusions and DNA copy number variants (CNVs)
    • More complex with RNA and DNA
    • Harder to source standards/positive patients due to low prevalence
  • Reevaluated reporting software to choose optimal platform that was capable of analyzing the addition of CNVs and Fusions.
## New Panel: Oncomine Focus Assay

### Hotspot genes, n=35

<table>
<thead>
<tr>
<th>DNA Panel</th>
<th>RNA Panel</th>
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<tbody>
<tr>
<td>AKT1</td>
<td>IDH2</td>
</tr>
<tr>
<td>ALK</td>
<td>JAK1</td>
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<tr>
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<td>JAK2</td>
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<tr>
<td>Braf</td>
<td>JAK3</td>
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<td>ROS1</td>
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<tr>
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### Copy Number Variants, n=19

<table>
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<tr>
<th>Fusion drivers, n=23</th>
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<tbody>
<tr>
<td>ALK</td>
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</table>

**52 unique genes**

**269 amplicons in DNA panel, 272 amplicons in RNA panel**
Oncomine Knowledge Reporter (OKR)

- Best-in-class interpretation
- Performed with a cloud-based software
- Much faster to generate a report
  - Reduced data analysis time from 20 minutes per case to 5 minutes per case.
  - Saves 5 hours per week of tech time!
- Clear and concise report
- Flexible to meet Oncologist’s needs
- Affordable
Current Validations

- Oncomine BRCA 1/2 Research Assay – 3 to 6 months from go-live
  - Two gene, two pool DNA panel
  - SNPs, INDELS, AND Large Genomic Rearrangements (LGRs)
    - LGRs span exon deletion/duplications, large INDELS, etc.
    - Samples sourced within one week by data mining our hospital networks EMR.
  - Commercial reference standards and patient DNA readily available.
  - Workflow optimized for automation from nucleic acid recovery to data analysis.
  - Reporting platform already selected.
Current Validations

• Oncomine Myeloid Research Assay
  • Have begun the validation on this assay.
  • Larger panel with fusions.
  • Panel optimized for nucleic acid extracted from fresh peripheral blood and bone marrow samples. FFPE embedded samples not recommended.
  • Commercial reference standards available.