Checklist for Bringing MDx Testing on Board

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Goals

- Operational considerations
- Regulatory considerations
- Reimbursement
Aims

Improved Diagnosis
Improved Predictors of Prognosis
Patient Management

Improved Selection of Therapeutic Modalities
Test Selection

- **Enhance cost-effective management of patient**
  - less expensive or effective method for diagnosis or overall care of patient

- **Director responsibility**
  - Send out list, perceive need by physician community,
  - TAT, technical capabilities, personnel expertise
  - patent issue
  - Professional Guidelines
    - CF guideline ACOG/ACMG
    - Fragile X testing
  - FDA approved/cleared tests
  - Reimbursement: All about CLINICAL UTILITY!!!
# Revenue center or and cost avoidance center?

## Reduce cost reference laboratory send outs

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Medicare expect</th>
<th>Cost reference lab</th>
<th>Prof/Loss send out</th>
<th>Total cost in house</th>
<th>Prof/Loss In house</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV viral load</td>
<td>98.07</td>
<td>128</td>
<td>-29.93</td>
<td>76.5</td>
<td>21.57</td>
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<tr>
<td>HCV viral load</td>
<td>46.29</td>
<td>275</td>
<td>-228.71</td>
<td>76.5</td>
<td>-30.21</td>
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<tr>
<td>HBV viral load</td>
<td>46.29</td>
<td>419</td>
<td>-372.71</td>
<td>76.5</td>
<td>-30.21</td>
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<tr>
<td>CMV viral load</td>
<td>46.29</td>
<td>265</td>
<td>-218.71</td>
<td>25.3</td>
<td>20.99</td>
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<tr>
<td>BKV viral load</td>
<td>27.02</td>
<td>363</td>
<td>-335.98</td>
<td>25.7</td>
<td>1.32</td>
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<td>EBV viral load</td>
<td>27.05</td>
<td>300</td>
<td>-272.95</td>
<td>23.47</td>
<td>3.58</td>
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<tr>
<td>HIV Geno</td>
<td>324</td>
<td>657</td>
<td>-333</td>
<td>156</td>
<td>168</td>
</tr>
<tr>
<td>HCV Geno</td>
<td>324</td>
<td>587</td>
<td>-263</td>
<td>87</td>
<td>237</td>
</tr>
<tr>
<td>Total</td>
<td>939.01</td>
<td>2994</td>
<td>-2054.99</td>
<td>546.97</td>
<td>392.04</td>
</tr>
</tbody>
</table>
Test formats

- Developed by IVD manufacturer- FDA approved or cleared
- Developed by IVD manufacturer- RUO
- Laboratory Developed Procedure (LDP)
  - Manual
  - Automated
Important considerations in MDx

A timely and accurate test result

Verification and validation
Quality control
Proficiency testing
Assay Verification

- Unmodified FDA-approved
  - Laboratories perform “assay verification” to ensure that the analyte(s) meet the manufacturer performance specifications before reporting patient results
    - Accuracy
    - Precision
    - Reportable range of test results
    - Verify appropriate reference intervals (normal values) for laboratory patient population
Analytical Validation

- Applies to
  - LDTs
  - Modified FDA approved tests

- Before reporting patient results, the laboratory must establish performance characteristics for
  - Accuracy
  - Precision
  - Reference and Reportable ranges
  - Analytical sensitivity (LOD and LOQ)
  - Analytical specificity
  - Interfering substances
  - Diagnostics (Clinical) Validity
Assay Validation cont...

- An extensive process
- LDTs: requires assay design, development, optimization and validate the performance characteristics
- ASRs: examine the components of a test that are assembled for the intended use without regulatory approval
- Establish the analytical performance and clinical (diagnostics) performance of a test as it applies to its intended use.
Guidelines for MDx-CLSI (www.nccls.org)

- MM01-A Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline
- MM02-A2 Immunoglobulin and T-Cell Receptor Gene Rearrangement Assays; Approved Guideline - Second Edition
- MM03-A2 Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline
- MM05-A Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline
- MM06-A Quantitative Molecular Methods for Infectious Diseases; Approved Guideline
- MM07-A Fluorescence In Situ Hybridization (FISH) Methods for Medical Genetics; Approved Guideline
- MM09-P Nucleic Acid Sequencing Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Proposed Guideline
- MM10A-Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline
- MM11P-Molecular Methods for Bacterial Strain Typing; Proposed Guideline
- MM12-A-Diagnostic Nucleic Acid Microarrays; Approved Guideline
- MM13-A- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline
- MM14-A-Proficiency Testing (External Quality Assessment) for Molecular Methods; Approved Guideline
- MM16-P- Use of External RNA Controls in Gene Expression Assays; Proposed Guideline
ACMG- (www.acmg.net)

- **Prenatal Diagnosis**

- **Fragile X**

- **Cystic Fibrosis**

- **Factor V Leiden**

- **FISH**
Pre-Validation considerations
Design and optimization of LDTs

- Correct target (HCV 5’non-coding region, BKV VP2, VP3 or Large T Antigen are highly conserved, etc.)
- Stringent design/analysis of primers and probes (previous published sequences, specific software, commercial vendors, NCBI genome database, etc.)
- Nucleic acid extraction method manual or automated (quality and quantity of extracted nucleic acid)
- Appropriate platform (chemistry) for the test
  - PCR
  - Southern Blot
  - Sequencing
  - Real-time PCR (TaqMan, Beacons, FRET)
  - Hybridization (bead arrays, CGH)
  - Microarrays
Pre-Validation considerations for the design of LDTs, cont...

- **Calibrators and controls**
  - Reference materials (WHO HIV, WHO HCV, NIST for Fragile X syndrome and Short Tandem Repeats)
  - Commercial controls (BBi, Acrometrix, Zeptometrix, etc.)
  - In house developed controls and calibrators
  - Coriell Repository
  - Samples Exchange

- **Optimization of amplification and detection**

- **Results interpretation**
Reference Materials

- WHO HIV, HCV, HBV, CMV, EBV
- NIST:
  - Fragile X syndrome Reference Material
  - Short Tandem Repeat database
  - DNA Standard Reference Material
- Commercial Sources
  - Seracare
  - Accrometrix
- Corriell Repository
- Samples exchange
Validation of LDT: Phase 1: Define intended use, set minimal acceptable performance

- Clinical indications: target population and reasons for ordering the test (diagnosis, prognosis, prediction, screening, monitoring, confirmatory)
- Specimens type (blood, paraffin, plasma, etc.) collection and handling procedures
- Clinical utility: risks and benefits of using the test in clinical practice; how the test improves the outcome of patient care
- Expected performance characteristics
- Design validation studies: MM-17P
Validation of LDT Phase 2: Generate validation data, optimize, and write validation report

Specific issues with multiplex assays

- Specimen types
- Analytical sensitivity (LoD):
  - Input range for each target and overall
  - Allelic dropout
- Analytical specificity
  - Interference and admixture
  - Analytical reactivity/cross reactivity
- Precision
  - multiple replicates of simulated samples representing all outcomes for each analyte probe
    - Repetability: under the same condition intra and inter run, same reagents, same tech, etc.
    - Reproducibility, vary techs, instruments, lots, days, etc.
Accuracy

• Analyze known samples (e.g. controls or reference materials) and compare results with that obtained by a reference method

• For genetics uncommon diseases, it may be necessary to obtain commercially available reference materials or controls from other laboratories

• The appropriate number of specimens depends on many factors including, but not limited to complexity of the assay, relevance of targets/alleles, etc.
Analytical Sensitivity

- Lowest amount of analyte that can be detected >95% of times tested
- *Can be done by*...
  - Control material of known concentration or copy number (calibrators/standards)
  - Dilutions of analyte (microorganism, gene) of known quantity
  - Genomic diversity: set lower limit of detection for each genomic variant
  - Quantified amount of RNA or DNA
Relationship between LOD and LOQ

Specificity

- Search Genbank or other comprehensive nucleic acid databases for similarity between sequences

- Perform nucleic acid detection studies on well-characterized isolates and strains
Clinical validation

- Systematically review the medical literature
  - Assays with well established clinical validity and utility where there is establish associations with clinically relevant phenotypes
    - Assay comparison including patient samples from the assay’s intended use population
  - Assay based on multivariate classifiers
    - Multivariate classifier, index, score using an empirically derived algorithm derived from a data set (training data set) should be clinically validated on a independent data set

- Conduct studies to sufficiently evaluate
  - Clinical sensitivity
  - Clinical specificity

- Clinical utility!

Elements of report

- Type of procedure
  - PCR, RT-PCR, QPCR, FISH, sequencing, etc.
- Defined target
  - name of gene or locus tested; use standardized gene nomenclature
- Pertinent details of procedure
  - ASR or kit name and version with name of manufacturer, instrument type
    - Reference range
      - normal versus abnormal
    - Interpretation section:
      - Analytic interpretation of result
      - Clinical interpretation of result
- Disclaimer on non-FDA approved tests in which a commercial analyte-specific reagents (ASRs) were used.
Standards Gene Nomenclature

- **Standard can change**
  - Common mutations that were designated using the method that was standard at the time of their identification is no longer considered to be correct
    - For example *CFTR* mutation commonly known as delta F508 (commonly used colloquial nomenclature) is properly designated as c.1521_1523delCTT or p.Phe508del
    - *BRCA2* mutation commonly known as 6174delT is correctly designated as c.5946delT, p.Ser1982Argfs*22

- **Universally understandable in the clinical community**
  - Allows correlation of test results b/w labs and reports in the literature

- **Important for accurate communication of test results to health care providers**

- **Recommended by ACMG and CAP etc.**
Human Genome Organization (HUGO) Gene nomenclature Committee (HGNC) 
AND
International Federation of Human Genetics Societies (IFHGS)

Under the auspices of

Human Genome Variation Society (HGVS)
Defines gene variation nomenclature
http://www.hgvs.org/mutnomen/index.html

HGVS has established guidelines for mutation nomenclature
The HGVS website provides detailed guidance in naming mutations
(www.hgvs.org/mutnomen/index.html)
HGVS nomenclature guidelines are revised as needed – it is recommended that the guidelines are reviewed periodically to verify current nomenclature guidelines
Documents, records and sample retention

- Requisitions
  - Consent for genetic testing
  - Family history
  - Privacy of medical records
- Record keeping
- Retention of NA
Important considerations in MDx

A timely and accurate test result

- Verification and validation
- Quality control
- Proficiency testing
Laboratory Space

- Pre-Amplification Laboratory (clean room)
  - Storage of chemicals and preparation of reagents
  - Processing specimens
  - Set up reactions

- Post-Amplification Laboratory
  - Amplification and detection
  - Capillary electrophoresis and other detection systems
  - DNA sequencing
MDx Personnel
CAP Molecular Pathology Checklist (www.cap.org)

- **Section Director/Technical Supervisor**
  - Pathologist, certified physician other than pathologist, doctoral scientist in biology science
  - with specialized training and/or appropriate experience in Molecular Pathology

- **Bench testing Supervisor**
  - Person who qualifies as a director/technical supervisor
  - CLSp(MB), BS, BA or MT (ASCP) with at least 4 years of experience (at least 1 year in molecular pathology under a qualified director)

- **Person performing technical aspect**
  - MT (ASCP) certified or equivalent
  - Experienced in the field under direct supervision of qualified director
  - BA or BS in biologic sciences w/ experience in molecular pathology methods
MDx Requirements

- **Structure:**
  - a) Clinical component
    - medical technologists dedicated to performing clinical assays and new procedures from the research protocols that are currently being performed by research component
  - b) Research and Development
    - Ph.D. level research scientist doing applied or basic research utilizing techniques that will be needed for clinical assays
Challenges in quality Management in Molecular Diagnostics

- Technology driven
- Transition from research to clinical laboratories
- Rapid evolution and change of technology and applications; emerging pathogens; new mutations and associations with disease
- Paucity of material for validation and standardization
- Non-uniformity of calibrators and controls
QC monitors are selected to assess

- Pre-analytical process
  - Specimen integrity (DNA, RNA)
- Analytical process
  - Sample prep, amplification, detection
  - Calibrators and controls
- Post-analytical process
  - Documentation, interpretation, reporting
QC Pre analytical level

- Requisition forms
  - Ethnic information and consent for genetic testing
- Specimen collection
  - Test specific tubes and containers
  - Storage specs until transport
- Specimen transport and storage
  - Temperature, conditions
- Specimen handling/processing
  - Speed and temp of centrifugation
QC at the analytical level

- Proficiency testing
  - CAP Proficiency surveys
  - Alternative Assessment
    - 2 x year 3-5 samples
- Use of positive, negative and sensitivity controls
- Controls with known values for quantitative tests
- Use internal, external, extraction and endogenous controls (inhibitors)
- Contamination control
Special Considerations Stratified by Clinical Application

• Genetics
  – Ethical issues (presymptomatic and prenatal testing)
  – Consequences for family members
  – Informed Consent Requirements
  – Requirements for family-related information (ethnicity, pedigree, specimens)
  – Time sensitivity
  – Complex risk assessment calculations
Oncology
- Many type of samples
- Extensive use of paraffin-embedded tissue samples (familiarity with limitations)
- Need for diagnostic samples for optimal interpretation of minimal residual disease test results
Special Considerations Stratified by Clinical Application

- Infectious Disease
  - High volume testing
  - Use of automated platforms
  - High cost of commercial in vitro diagnostics test kits
  - Increased need for quantitative testing with a wide dynamic range and low limit of detection
Questions?