The Role of Quality Assurance in the Molecular Laboratory

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Agenda

• Is there a need for Quality Assurance in Molecular Testing?

• Molecular External Quality Assessment

• ISO 15189 – Quality Testing

• Validation / Verification
Molecular Diagnostics

• Traditional & more advanced testing

≈ 2300 genetic inherited disorders testing offered in clinical labs

• Rapid development of drug treatments that are targeted to molecular and protein anomalies of the tumor
  – Diagnostic tests
  – Tissue / FFPE (HER2, EGFR, KRAS etc...)

Dublin Pathology 2015
Why is there a need for Quality Assurance in the Molecular Lab?

- Challenges at each step
- Fixation = sub optimal DNA/RNA
- Standardization of test and sample
  - Pre examination
  - Examination
  - Post examination
Why is there a need for Quality Assurance in the Molecular Lab?

- High Quality Testing & Lab Performance is required
- Both internal & external quality assurance (PT)
- Controls
- Competency of Personnel & Training
• International Survey

• 74% Participate in PT/EQA
• The aim of External Quality Assessment programs
  – monitor and improve quality within the clinical laboratory by assessing a laboratory’s ability to use molecular diagnostic technologies within the routine clinical setting.

• The EQA programs support the clinical laboratory’s
  – regulatory requirements
  – educational in application.
• The reports and practical feedback should provide participant to:
  – identify and resolve potential problems
  – monitoring the effectiveness of their laboratory quality assurance processes.

• Improves by identifying inaccuracies that a lab can trace errors in their processes
Over 12 Global Molecular EQA Schemes
Cytogenetics
Biochemical Genetics
Molecular Genetics
Pharmacogenetics
Inherited Metabolic genetic Diseases
Molecular Oncology – Solid Tumor
Multigene Tumors

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- Cytogenetics
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- Molecular Genetics
- Pharmocogenetics
- Inherited Metabolic genetic Diseases
- Molecular Oncology – Solid Tumor
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- InSitu Hybridization
- RT PCR
- Sequencing
- DNA Amplification
- Gene Rearrangement
- NGS
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Microarray
Digital Images
FFPE
Samples
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InSitu Hybridization
RT PCR
Sequencing
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NGS

Microarray
Digital Images
FFPE
Samples

Counting
Staining
Scoring
Interpretation Reporting
Graph displaying the different methodologies used by participating laboratories in 2011 runs 1 and 2.

30 countries - 72% passed
correct identification of all genotypes by a given laboratory in all the 3 years was only 40%
Methods Used to Test for Inherited Disorders

<table>
<thead>
<tr>
<th>Method Used</th>
<th>% of Diseases Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing(^a)</td>
<td>93 (90 of 97)</td>
</tr>
<tr>
<td>Transcription-mediated amplification</td>
<td>23 (22 of 97)</td>
</tr>
<tr>
<td>Deletion/duplication analysis</td>
<td>27 (26 of 97)</td>
</tr>
<tr>
<td>Mutation scanning</td>
<td>18 (18 of 97)</td>
</tr>
<tr>
<td>Methylation analysis</td>
<td>2 (2 of 97)</td>
</tr>
</tbody>
</table>

\(^a\) Analysis of a random sample (~10%) of diseases for which molecular genetic testing methods are used (February 26, 2009 report of ~970 diseases obtained from GeneTests), determined that testing for ~93% (90 of 97) of these diseases used DNA sequencing methods in at least some of the laboratories that offered testing; 49% (48 of 97) of the diseases in the sample were tested using only sequencing techniques.
Percent of problems in the lab

- 20-30% Error rate
- Genotyping reporting errors
- Need of a common mutation nomenclature
- Amount of tumor being analyzed
- Heterogeneity in the sample
• Challenges should mimic clinical samples and encompass all steps of the testing process (ISO 15189)

• EQA Programs inform labs of poor performance
  – Directly advise national/regional accrediting body
  – Majority correct deficiencies before reaching continuous poor performance
Auditors are our friends.
ISO 15189 : 2012

Quality Testing
Quality Management

- Ongoing effort that includes policies and procedures established and implemented for the purpose of providing accurate lab results
ISO 15189 Quality Control

• Quality Control 7.3.2

• ISO requires that ‘the laboratory shall design a quality control procedures that verify the attainment of the intended quality of results”
  – Define the quality requirements
  – Determine the method precision and bias
  – Identify procedure
  – Predict performance
  – Set goals for performance
  – Select an appropriate procedure
ISO 15189 Quality Control

• Quality Control Data 7.3.2.2

• Requires the laboratory shall have a procedure to prevent the release of patient results when IQC data indicates a problem.

• 4.9 Indication and Control of Nonconformities

• Quality Control Rules are violated
  – patient samples should be examined
ISO 15189  Inter Laboratory Comparisons

- The results of the testing laboratory are compared with those of an approved reference laboratory.

- ISO 17043: Proficiency Testing (PT) evaluation of participant performance against pre-established criteria by means of inter laboratory comparisons.

- European Standard EN14136: External Quality Assessment (EQA) determination of individual and collective laboratory performance and performance characteristics, of examination procedures by means of inter laboratory comparisons.
ISO 15189  Inter Laboratory Comparisons

- ISO requires that a lab should participate in inter laboratory comparison programs: examples EQA or PT programs
EQA Participation Procedure Contents

5.0 PARTICIPATION

5.1 Reception of EQA items

5.1.1 Reception of EQA items and appropriate storage

5.1.2 Preparation prior to use if required

5.2 EVALUATION of EQA ITEMS

5.2.1 Examination of EQA items

5.2.2 Recording of results

5.3 DISPATCH OF EQA RESULTS

5.3.1 Checking of results to be returned

5.3.2 Dispatch of EQA results to the EQA scheme

5.4 REVIEW of EQA REPORT

5.4.1 Review of EQA results and recording of any nonconformities or potential nonconformities

5.4.2 Record of review and corrective or preventative action.
## EQA PARTICIPATION FORM

To be used to monitor participation in EQA schemes

<table>
<thead>
<tr>
<th>RECORD FILENAME</th>
<th>QF-GEN-EQP1 Part# NEQAS Gen 04/06/2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEPARTMENT</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>SECTION</td>
<td>Routine chemistry</td>
</tr>
<tr>
<td>SECTION SUPERVISOR</td>
<td>Joe O'Brien</td>
</tr>
</tbody>
</table>

### 5.1 RECEPTION of EQA Items

<table>
<thead>
<tr>
<th>EQA scheme name</th>
<th>NEQAS Gen</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQA Item Identification(s)</td>
<td>NG-A001, NG-A002, NG-A003</td>
</tr>
<tr>
<td>Date item(s) received*</td>
<td>04/06/2012</td>
</tr>
</tbody>
</table>

### 5.2 EXAMINATION of EQA items

| Date items examined | 21/06/2012 |

### 5.3 DISPATCH of EQA results

| Results prepared by/checked by | David McCall/Joe O'Brien |
| Date results dispatched (copy attached) | 21/06/2012 |

### 5.4 REVIEW of EQA report

| Date report received (copy attached) | 04/07/2012 |
| Report discussed with (name/group) | David McCall and routine Biochemistry staff |
| Date report discussed | 06/07/2012 |

### Actions to be taken

<table>
<thead>
<tr>
<th>Observations</th>
<th>Action to be taken by whom</th>
<th>Target date for completion</th>
<th>Nonconformity No. (discharge date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ca++ result on NG-A001 outside quality goal</td>
<td>Check original result and result returned/David McCall</td>
<td>07/07/2012</td>
<td>NC/2011/997 (07/07/2012)</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. etc</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Please note here if there is discrepancy between date received and expected date of receipt.
Validation/Verification
Validation/Verification

* Ensure standards of laboratory practice and accuracy of tests results generated meet the intended application.
Verification

• FDA & ISO definition:

• “Confirmation through the provision of objective evidence that specified requirements have been fulfilled”

• Replicate the manufactures performance claim - package insert

• End users patient population specimen mix

• Applies to non modified tests
Validation

- FDA & ISO Definition:
  - “Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use can be consistently fulfilled”

- World Health Organization (WHO)
  - The action of proving procedure, process system works as expected to achieve intended result

- EU IVD Directive 98/79/EC
  - Includes not only test but test system
Validation

- Deviate from the manufacturers package insert
- Laboratory Developed Tests
  - Performance characteristics must be established
Workflow for Implementing a new molecular test or test system in the routine diagnostic laboratory

- Validation work (ISO 9001:2008; ISO 15189)
- Implementation of a new molecular test or test system
- Verification Work

**IVD/CE labeled /FDA approved**
- Accuracy
- Imprecision
- Analytic measuring range
- Recovery (interference testing)

**Laboratory Developed, etc.**
- Accuracy
- Imprecision (within & between runs)
- Analytic measuring range
- Recovery (interference testing)
- Reproducibility
- Specificity
- Lower limit of detection/quant
Components required for validation of molecular tests or test systems according to ISO 9000 and ISO 15189

Component required

- Quality control: internal controls, external run controls, international standards and reference materials
- Proficiency testing participation for comparison of inter-laboratory test results
- Validation of employee competency
- Instrument maintenance and calibration
- Correlation with clinical findings

Particular requirements

- Quality control records including performance data, instrument printouts, and corrective action
- Proficiency testing results including corrective actions
- Key and operating staff qualifications, credentials verifying field expertise
- Instrument records including printouts of maintenance and calibration protocols
- Diagnostic sensitivity and specificity of the test or test system
Conclusion

- Continual quality improvement is essential in providing exceptional patient results.
- EQA improves the quality of testing by identifying inaccuracies and can trace these to test processes.
- Validation and Verification provide assurance that the test system is performing as intended.
#5 Continuous Improvement
Thank You!