DNA quality assessment – what can we learn from our neighbours

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Institute of Clinical Chemistry
1. Introduction

2. Molecular genetic EQA schemes of the RfB

3. FV

4. Conclusion

5. Future Development
Introduction

- External quality assessment schemes – definition and aim
- Unique characteristics of molecular genetic diagnostics to be considered
- Database concerning EQA provider: [www.europgentest.org/](http://www.europgentest.org/)

<table>
<thead>
<tr>
<th>Assay</th>
<th>EQA Provider</th>
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<td>Cytochrom p450 2B6*6</td>
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<td>RfB, ECAT</td>
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<td>Cytochrom p450 2C8 (CYP2C8) Gene: K399R</td>
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<td>Cytochrom p450 3A5*3</td>
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<td>DNA Isolation</td>
<td>RfB, ECAT</td>
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<td>DNA Sequencing</td>
<td>RfB, CAP, ECAT, EMOQ, EQUALIS</td>
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</table>
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Molecular genetic proficiency testing of the RfB

Scope:

- DI – DNA Isolation
- FV – Genotyping
- SQ – Sequencing
DI-EQA

- Distribution twice a year by the „Referenzinstitut für Bioanalytik“ ([www.dgkl-rfb.de](http://www.dgkl-rfb.de))
- Offered since 2009
- Two different samples each á 500μl of human whole blood are provided
- Following parameters are requested:
  - Method used for DNA-isolation
  - DNA-concentration
  - Purification of the isolated DNA
- Following analytes can be determined:
  - FV-Leiden, FV-Hong-Kong, FV-Cambridge
  - Since 2012 MTHFR 677, FII, HFE
DI-EQA

1. FV-Leiden (ARG506GLN)
   - Allele: R:R506, Q:Q506
     - Probe/Sample
       - R/R: 01, 1; 02, 41
       - R/Q: 01, 1; 02, 1
       - Q/Q: 01, 39

2. FV-H1299R (HIS1299ARG)
   - Allele: H:H1299, R:R1299
     - Probe/Sample
       - H/H: 01, 5; 02, 6
       - H/R: 01, 6; 02, 1
       - R/R: 01, 1; 02, 1

3. FV-Cambridge (ARG306THR)
   - Allele: R:R303, T:T306
     - Probe/Sample
       - R/R: 01, 3; 02, 3
       - R/T: 01, 3; 02, 3
       - T/T: 01, 3; 02, 3

4. FV-Hong-Kong (ARG306GLY)
   - Allele: R:R306, G:G306
     - Probe/Sample
       - R/R: 01, 3; 02, 3
       - R/G: 01, 3; 02, 3

- Your results are marked with a green dot -

Used methods:
automated: 26
manually: 16

Quotient 260/280 - Sample A
Quotient 260/280 - Sample B

amount of DNA added in PCR - Sample A
amount of DNA added in PCR - Sample B

DNA isolated from 1μl blood
SQ-EQA

- Distribution twice a year by the „Referenzinstitut für Bioanalytik“ ([www.dgkl-rfb.de](http://www.dgkl-rfb.de))
- Offered since 2006
- Two different PCR-products as well as the sequencing primers are provided
- Separated into a technical and medical part
- Technical part:
  - Both samples have to be analyzed
  - The raw data has to be edited
- Medical part:
  - The patients history as well as other laboratory findings are provided
  - Only one sample has to be evaluated
SQ-EQA

DGKL EQA for DNA-sequencing
- Based on EQUAL-Seq und published reporting formats

Agenda

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• Distribution twice a year by the „Referenzinstitut für Bioanalytik“ (www.dgkl-rfb.de)
• Offered since 2002
• Samples containing 500 to 1000 ng of lyophilized gDNA
• Since 2014 nine different sets (A-I) are provided
  • Each set is composed of two different samples
  • An average of four different analytes are provided per set
  • For sample validation two different methods are used
FV-EQA: scope

Number of participating laboratories

X 2.5
FV-EQA: scope

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<tr>
<th>Countries</th>
<th>Number of Participants</th>
<th>Number of Participants (%)</th>
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<td>35.1</td>
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<td>Austria</td>
<td>34</td>
<td>9.1</td>
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<tr>
<td>Swiss</td>
<td>28</td>
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<td>France</td>
<td>39</td>
<td>10.5</td>
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<tr>
<td>Benelux</td>
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<td>10.5</td>
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<tr>
<td>Other Europe</td>
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<td>North America</td>
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</table>

Increment of participating laboratories in the last two EQAs compared to 2002
FV-EQA: scope

Number of analytes offered
**FV-EQA: scope**

**Set A:** FV-Leiden, Prothrombin, MTHFR (C677T, A1298C), PAI-I 4g5g

**Set B:** FXIII V34L, GPIIIa, βFib g-455a, VKORC1 (g-1639a/c1173t), FXII c-46t, FV H1299R

**Set C:** a1 PI, Apo E, Apo B100, ACE, CETP

**Set D:** TPMT, Cyp2C19 *1/*2/*17, Cyp2C8 (K399R), Cyp2C9 *2/*3, UGT1a1 (*28), DPD Exon 14 skipping, BCHE A/K

**Set E:** ALDO B (149/174/334), HFE (H63D, C282Y, S65C), LCT c-13910t, NOD2 (R702W, G908R, L1007fins C)

**Set F:** M. Wilson ATP7B-C3207 A, FSAP (Marburg-I), ITGA2 Gplalla C807T, Col1A1 SP1, VDR (BsmI, Apal, TaqI)

**Set G:** K-Ras: Codon 12/13/61, BRAF V600E

**Set H:** HLA-B27

**Set I:** Cyp2D6
FV-EQA: scope

Number of genotypes determined
FV-EQA: scope

Number of genotypes determined per analyte
FV-EQA: methods

- TaqMan-probes; 2043; 10%
- AS-amplification; 1711; 9%
- reverse Dot-Blot; 1966; 10%
- RFLP; 2040; 10%
- DNA Sequencing; 1657; 8%
- other; 3153; 16%
- Pyrosequencing; 872; 5%
- home-brew method; 453; 2%
- FRET-probes; 5640; 29%
- Chip-Analysis; 135; 1%
- Molecular Beacons; 28; 0%
FV-EQA: error rate

Mean error rate (%)
FV-EQA: error rate

Mean error rate for each analyte
FV-EQA: error rate

Error rate depending on method used

- **RQ-P**
  - Number of errors: 57
  - Number of genotypings: 2384

- **AS-amplification**
  - Number of errors: 24
  - Number of genotypings: 2148

- **reverse Dot-Blot**
  - Number of errors: 39
  - Number of genotypings: 2408

- **FRET-probes**
  - Number of errors: 93
  - Number of genotypings: 6708

- **TaqMan-probes**
  - Number of errors: 29
  - Number of genotypings: 2906

- **Molecular Beacons**
  - Number of errors: 0
  - Number of genotypings: 62

- **other**
  - Number of errors: 35
  - Number of genotypings: 2008

- **DNA Sequencing**
  - Number of errors: 30
  - Number of genotypings: 2682

- **Chip Analysis**
  - Number of errors: 17
  - Number of genotypings: 430

- **Pyrosequencing**
  - Number of errors: 6
  - Number of genotypings: 1212

- **home-brew method**
  - Number of errors: 15
  - Number of genotypings: 712
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<th>FV-EQA: error rate</th>
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</table>
FV-EQA: error types

Evaluation of the FV1_09

Errors

– Reporting (e.g. LCT)
– Analytic
  ✤ by rare sequence variations, which result in non-standard genotyping (frequently specific for a genotyping method)
  ✤ Inadequate genotyping (e.g. CYP2D6)
FV-EQA: error types
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Conclusions

1. Increasing number of participants, analytes offered and analytes determined per laboratory
2. Changes in respect to the methods used for genotyping over the years
3. Identification of best-in-class methods
4. Reduction of the overall error rate by EQA scheme participation
5. Determination of inappropriate methods per genotype
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Future development

Separation of FV into MG1 and MG2:

- Analytes being included: FVII R353Q, AT3 Cambridge, CYP3A5*3, TNFalpha 238 and 308, HLA-B*5701, CYP2B6, IL28B C/T polymorphism, IL6, CYP3A4*22

New EQAs

- EQA scheme for isolation of circulating nucleic acids
- EQA scheme for NGS
Questions?