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Summary of key processes for tumor *BRCA* testing

Q&A Session

29.01.2018 Hadassah Medical Center, Jerusalem| Sabine Merkelbach-Bruse

Review of key processes

Overview

- Summary of key processes

- Quality assurance
  - internal QA
    - definitions: validation / verification
    - parameters for Quality assurance
    - example for validating *BRCA* testing
  - external QA
    - ring trials and guidelines

- Questions and Answers

Key steps in *BRCA* testing

- macrodissection of tumour tissue

- DNA extraction and estimation of concentration

- choosing the right system for parallel sequencing

- quality control during each run

- interpretation of results

- determining pathogenicity and reporting

Key steps in *BRCA* testing - macrodissection

- tissue blocks have varying tumour cell content, for NGS > 10 % of tumour cells are needed
- precise highlighting of the tumour area and estimation of tumour cell content has to be done by the pathologist on the H&E stained slide

marked slides and corresponding tissue blocks

cytological specimen with estimation of tumour cell content

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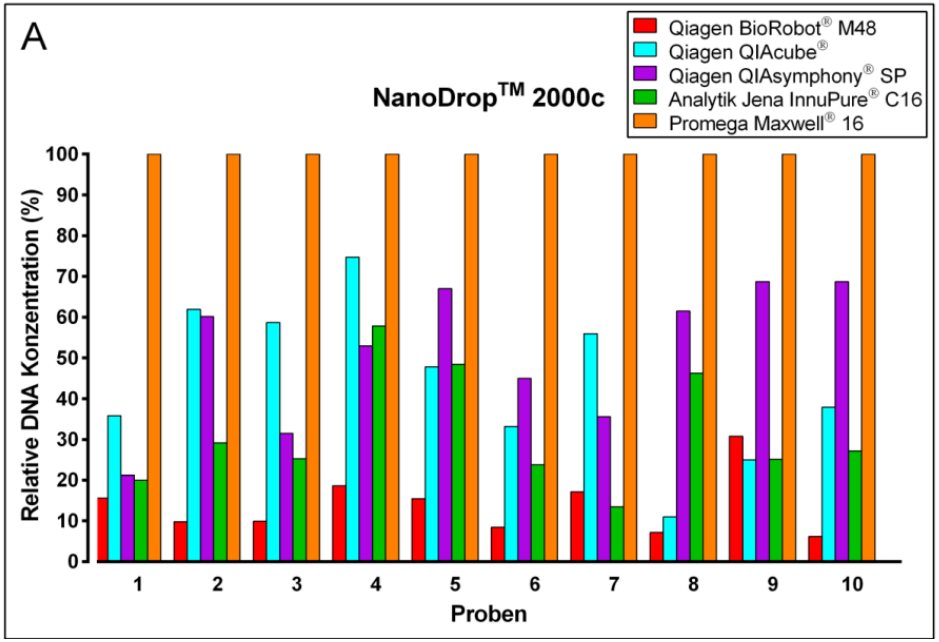
Key steps in *BRCA* testing – extraction and concentration

- many automated and manual systems are available
- DNA extraction method should yield good DNA quality and quantity and work well in downstream applications



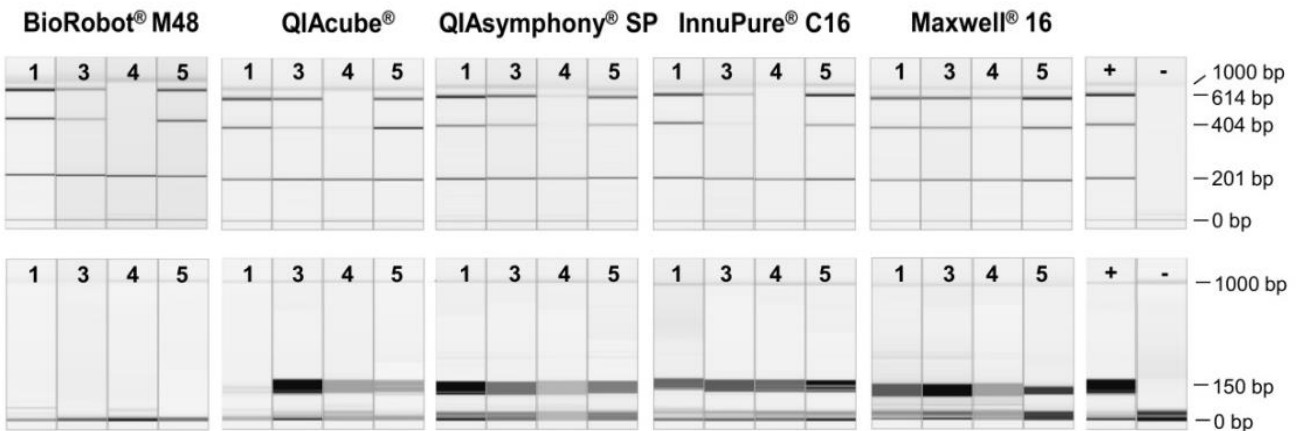
highest yield with **Promega Maxwell 16**

Heydt et al., Plos ONE, 2014

Works well with

PCR

Library preparation



Key steps in *BRCA* testing – parallel sequencing

- **MiSeq:**
  - high sequencing capacity
  - sequencing technology avoids homopolymer artefacts

- commercially available primer assays vs. lab developed primer assays

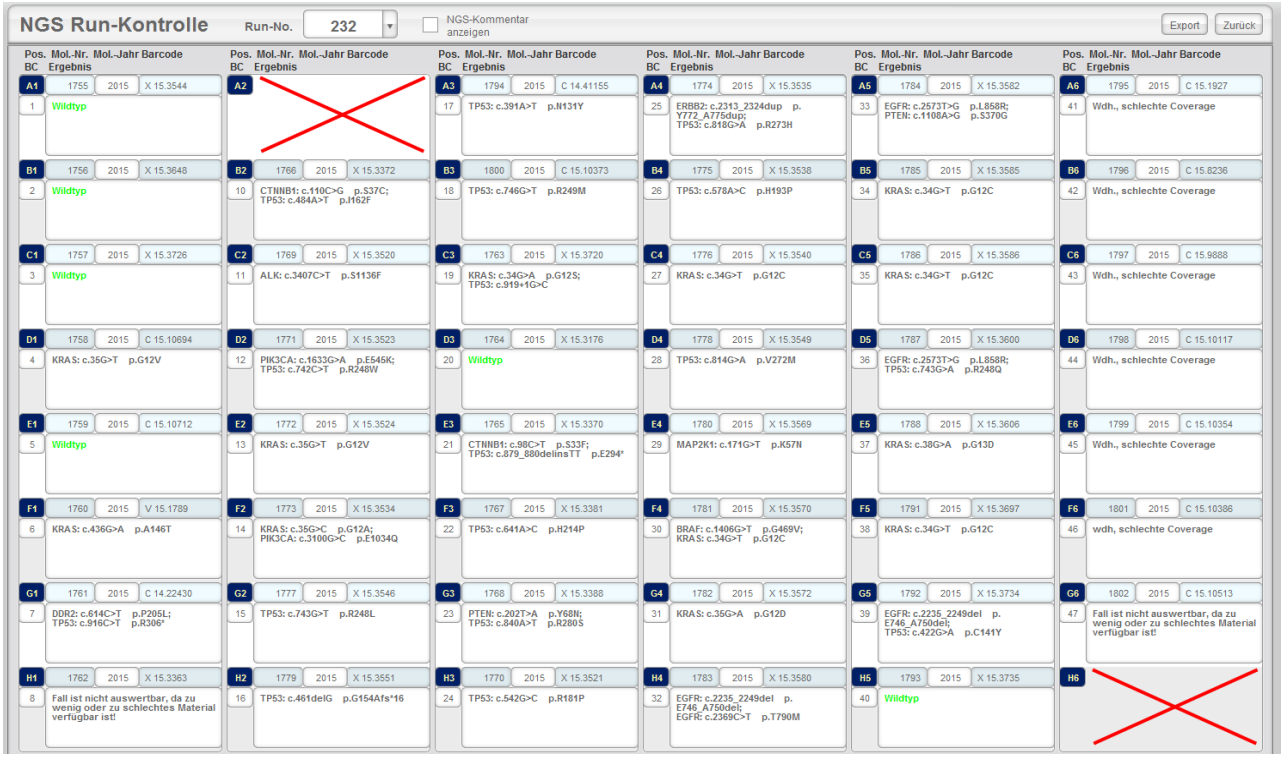
Advantages of commercial systems:	**Advantages** of LDTs:
- wet lab tested by manufacturer	- higher flexibility
- detailed protocols available	- combination with other assays
	- cheaper

Quality criteria for primer sets

- horizontal coverage = targeting regions of interest
- vertical coverage = read depth

Key steps in *BRCA* testing – quality control during run

- separation of pre- and post PCR working areas throughout the whole process
- negative control (without DNA) running alongside samples through library preparation and sequencing
- control of fragment size and concentration throughout library preparation
- correct handling of beads during purification steps
- barcodes should be changed between runs
- survey of the whole run to control for sample contamination



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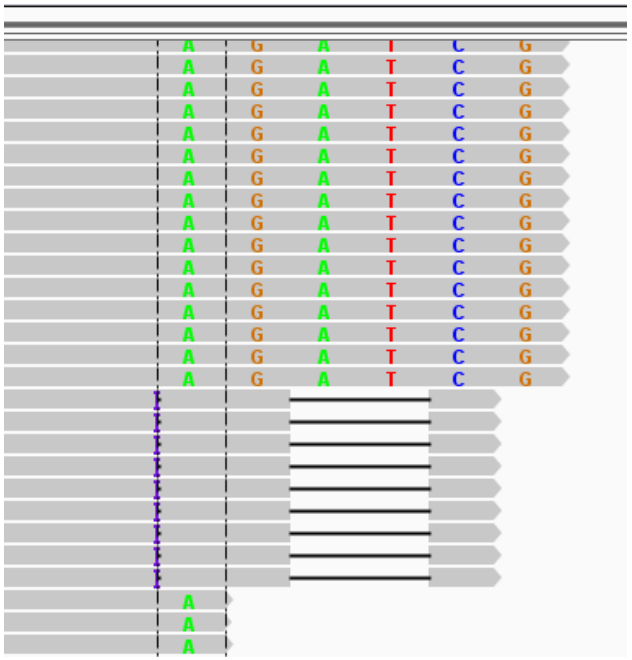
Key steps in *BRCA* testing – result interpretation

- the **vertical coverage** for each amplicon to be evaluated should be at least **200x**
- **allelic fraction** of reported mutations should be higher than **5%**

- all mutations should be controlled in the **IGV** to rule out
  - fixation artefacts
  - low stringency of primer trimming
  - wrong alignment



Key steps in *BRCA* testing – determining pathogenicity

- name true variants according to the rules of the human genome variation society
- check databases for variant classification, for example

ARUP: http://arup.utah.edu/database/BRCA/Home/BRCA1_Landing
UMD: http://www.umd.be/BRCA1/
IARC/LOVD: http://BRCA.iarc.fr/LOVD/home.php?select_db=BRCA1
ClinVar: http://www.ncbi.nlm.nih.gov/clinvar/?term= [brca1]

- use the rules of the ENIGMA consortium

ENIGMA *BRCA1/2* Gene Variant Classification Criteria

ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) is an international consortium of investigators focused on determining the clinical significance of sequence variants in breast cancer genes. Information about the consortium purpose, membership criteria and operation can be found at http://www.enigmaconsortium.org/.

Key steps in *BRCA* testing – reporting

Capoluongo et al., Seminars in Oncology

- targets analyzed
- the regions covered for each gene
- overall results: either pathogenic or deleterious variants present or absent
- mutation details: cDNA and amino acid change according to HGVS nomenclature
- reference sequence
- summary and interpretation

Statement:

….recommendation of ‘targeted therapy‘ if clinical indication is given and patient has a class 4 or 5 mutation

….if BRCA mutation is found in tumour and no germline data are available the report needs to clarify that there may be a germline mutation⇨ recommendation of genetic counseling

Quality control - definition of Verification and Validation

„Doing the test correctly or doing the correct test?“

Verification: Confirmation, through the provision of objective evidence, that **specified requirements** have been fulfilled

Validation: Confirmation, through the provision of objective evidence, that the **requirements for a specified intended use** or application have been fulfilled

- *in house (laboratory) developed tests (LDT) have to be validated*

- *commercially available tests only have to be verified (if they are used as specified)*

How is the new process validated?

- in Germany, many institutes of pathology are accredited according to DIN ISO 17020

Which parameters have to be determined?

Accuracy:

- precision
- correctness

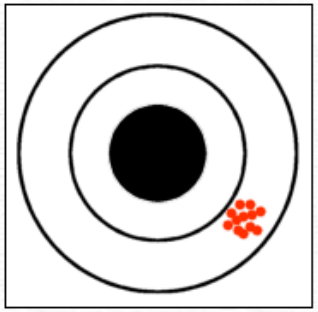
Selectivity:

- sensitivity
- specificity

Do all parameters comply with the quality requirements?

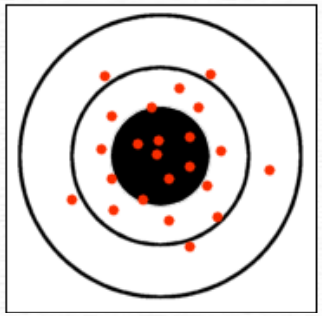
According to:
„Guideline [….] for the validation of examination methods in Molecular Pathology“ – DAkkS 71SD 4 037

Precision and Correctness – the same?



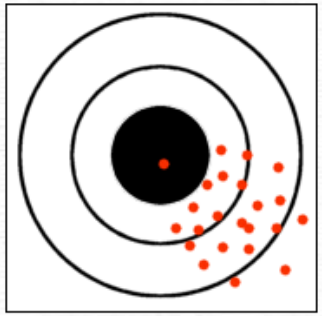
Precise, but incorrect

All shots are precise (nearly in the same region) but the shooter misses the center



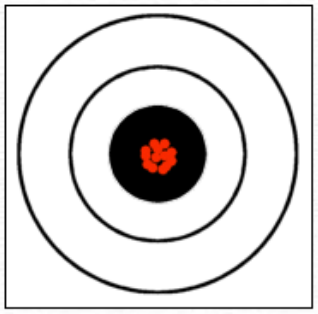
Correct, but imprecise

The shooter hits approximately the target, but the shots scatter



Incorrect and imprecise

The shooter hits the target only once and the shots scatter



Correct and precise

The shooter hits the target at all times

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Measurement of precision and correctness

Precision

- is the closeness of agreement among a set of results from the same sample

intra-assay precision (within run)

- each sample is measured several times under same conditions

inter-assay precision (day-to-day, batch-to-batch)

- the same samples are measured in different assays

Correctness

- is the closeness of a measurement to the true value (expected reference value)

- how is correctness determined?
  - comparison with results of a previously tested and validated method
  - measurement of an external reference sample cohort
  - use expectation values that are based on scientific results

Defining sensitivity and specificity

Sensitivity **„true positive rate“**

example: 5 (out of 100) sick people are tested as negative although having the condition: sensitivity 95%, 5% false negative

$$\frac{95}{95+5} = 95\%$$

Specificity **„true negative rate“**

example: 5 (out of 100) healthy people are tested as positive although not having the condition: specificity 95%, 5% false positive

$$\frac{95}{95+5} = 95\%$$

Example - Validation of *BRCA* Panel

Correctness

Samples: 55, results known from previous germline testing or Sanger sequencing

- 46/46 with concordance (100%)
- 9 cases couldn‘t be evaluated due to low sample quality

100%

Precision

Step 1

- five mutated samples
- triplicates of library prep
- all mutations identified

Step 2

- five mutated samples
- duplicates of library prep
- two different days, two different people
- two different runs
- all mutations identified

100%

Published Guidelines for Molecular Testing - NGS

Virchows Arch
DOI 10.1007/s00428-016-2025-7

REVIEW AND PERSPECTIVES

Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL

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Published Guidelines for Molecular Testing - NGS

College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests

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• *Context.*—The higher throughput and lower per-base cost of next-generation sequencing (NGS) as compared to Sanger sequencing has led to its rapid adoption in clinical testing. The number of laboratories offering NGS-based tests has also grown considerably in the past few years, despite the fact that specific Clinical Laboratory Improvement Amendments of 1988/College of American Pathologists (CAP) laboratory standards had not yet been developed to regulate this technology.

Objective.—To develop a checklist for clinical testing using NGS technology that sets standards for the analytic wet bench process and for bioinformatics or "dry bench" analyses. As NGS-based clinical tests are new to diagnostic testing and are of much greater complexity than traditional Sanger sequencing–based tests, there is an urgent need to develop new regulatory standards for laboratories offering these tests.

Design.—To develop the necessary regulatory framework for NGS and to facilitate appropriate adoption of this technology for clinical testing, CAP formed a committee in 2011, the NGS Work Group, to deliberate upon the contents to be included in the checklist.

Results.—A total of 18 laboratory accreditation checklist requirements for the analytic wet bench process and bioinformatics analysis processes have been included within CAP's molecular pathology checklist (MOL).

Conclusions.—This report describes the important issues considered by the CAP committee during the development of the new checklist requirements, which address documentation, validation, quality assurance, confirmatory testing, exception logs, monitoring of upgrades, variant interpretation and reporting, incidental findings, data storage, version traceability, and data transfer confidentiality.

(*Arch Pathol Lab Med.* 2015;139:481–493; doi: 10.5858/arpa.2014-0250-CP)

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Published Guidelines for Molecular Testing - BRCA

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Guidance Statement On *BRCA1/2* Tumor Testing in Ovarian Cancer Patients

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External Quality control

- any laboratory offering *BRCA* mutation testing should participate in external quality control assessments

  - Quality Assurance Initiative Pathology QUIP

  - Molecular Genetics Quality Network EMQN www.emqn.org

  - United Kingdom National External Quality Assessment Service UKNEQAS www.ukneqas.org.uk

  - College of American Pathologists CAP www.cap.org

External Quality control –reports on different ring trials

Virchows Arch (2016) 468:697–705
DOI 10.1007/s00428-016-1919-8

ORIGINAL ARTICLE

NGS-based BRCA1/2 mutation testing of high-grade serous ovarian cancer tissue: results and conclusions of the first international round robin trial

Volker Endris[1] · Albrecht Stenzinger[1] · Nicole Pfarr[1,8] · Roland Penzel[1] · Markus Möbs[2] · Dido Lenze[2] · Silvia Darb-Esfahani[2] · Michael Hummel[2] · Sabine-Merkelbach-Bruse[3] · Andreas Jung[4] · Ulrich Lehmann[5] · Hans Kreipe[5] · Thomas Kirchner[4] · Reinhard Büttner[3] · Wolfram Jochum[6] · Gerald Höfler[7] · Manfred Dietel[2] · Wilko Weichert[1,8] · Peter Schirmacher[1]

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RESEARCH ARTICLE

An evaluation of the challenges to developing tumor BRCA1 and BRCA2 testing methodologies for clinical practice

Gillian Ellison, Miika Ahdesmäki ✉, Sally Luke, Paul M. Waring, Andrew Wallace, Ronnie Wright, Benno Röthlisberger, Katja Ludin, Sabine Merkelbach-Bruse, Carina Heydt, Marjolijn J.L. Ligtenberg, Arjen R. Mensenkamp, David Gonzalez de Castro, Thomas Jones, Ana Vivancos, Olga Kondrashova, Patrick Pauwels, Christine Weyn, Eric Hahnen, Jan Hauke, Richie Soong, Zhongwu Lai, Brian Dougherty, T. Hedley Carr, Justin Johnson, John Mills, J. Carl Barrett

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What people think about during your conference talk

Thank you!!

Time for Questions!!