Defining Optimal Tissue Handling Procedures for Multiplex In Vitro Diagnostics

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Presentation build up

- Definition multiplex testing
- Tissue handling and discovery of genetic profiles
- Tissue handling and multiplex diagnostics
- Validation of FDA Cleared MammaPrint® tissue handling Procedures
- Summary

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What is Multiplex Testing (IVDMIA)

"An In Vitro Diagnostic Multivariate Index Assay (IVDMIA) is an assay that combines the values of multiple variables using an interpretation function to yield a single, patient-specific result (e.g., a "classification," "score," "index," etc.), that is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, and provides a result whose derivation is non-transparent and cannot be independently derived or verified by the end user."

Source FDA guidance document 1610.pdf July 26th 2007

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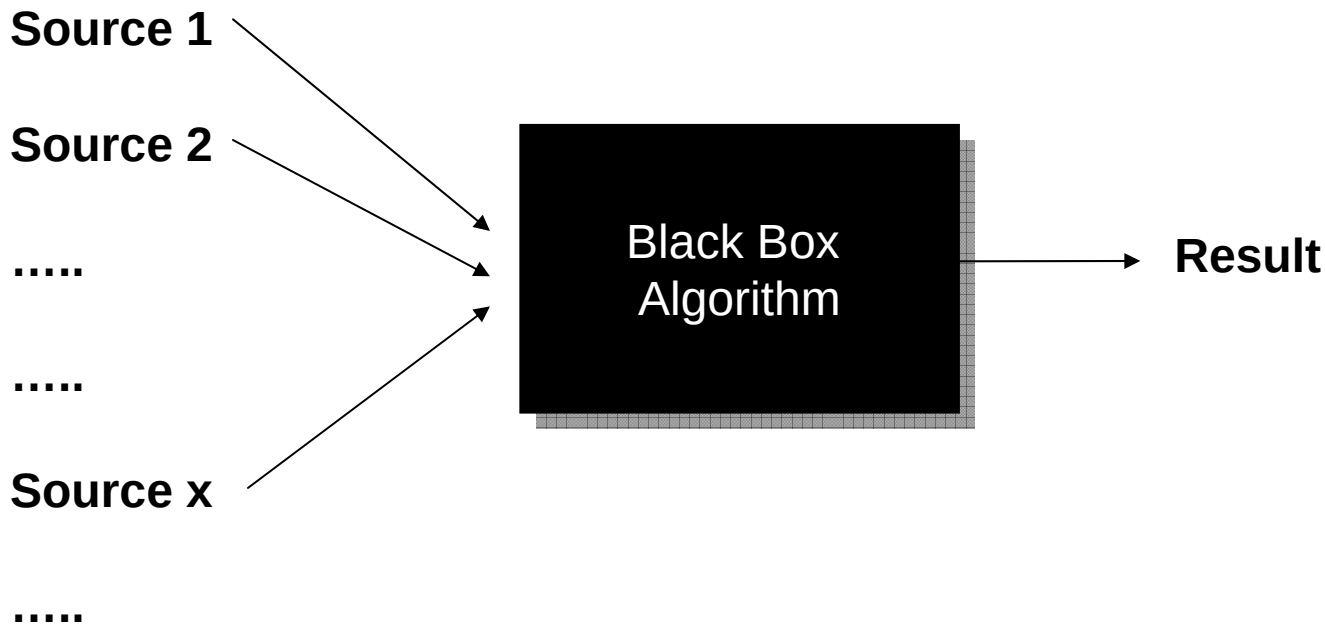
IVDMIA graphic representation



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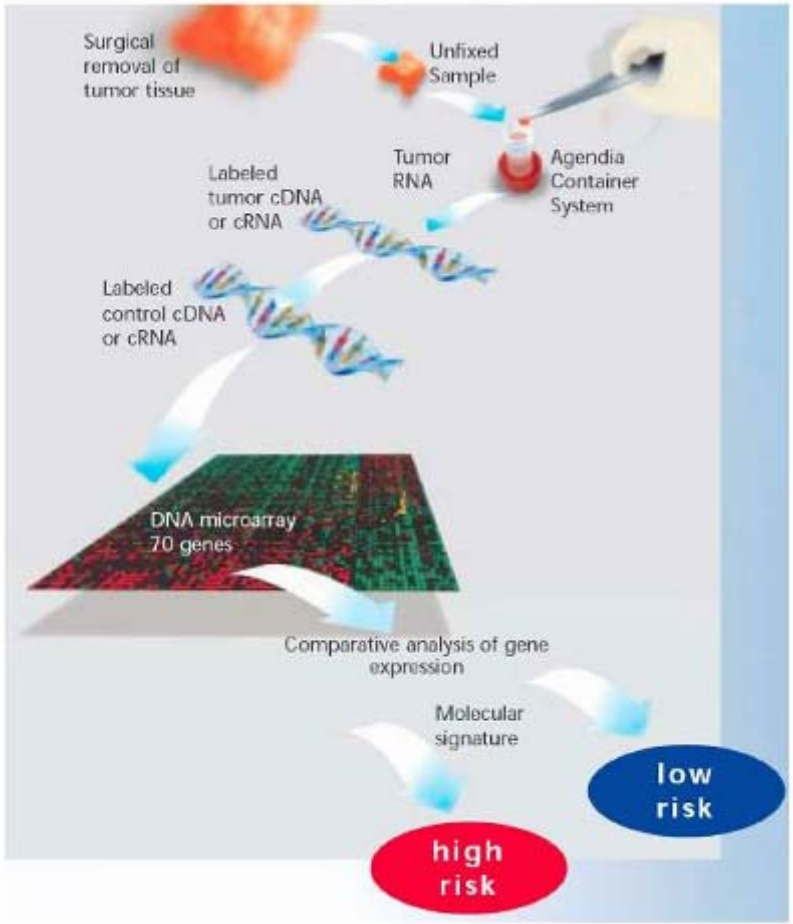
Multiplex test example- MammaPrint

MammaPrint is a qualitative in vitro diagnostic test service, performed in a central reference laboratory, using the gene expression profile of fresh breast cancer tissue samples to assess a patients' risk for distant metastasis.

Agendia is the first, and only one to date, to acquire FDA clearance for an IVDMIA (i.e., MammaPrint).



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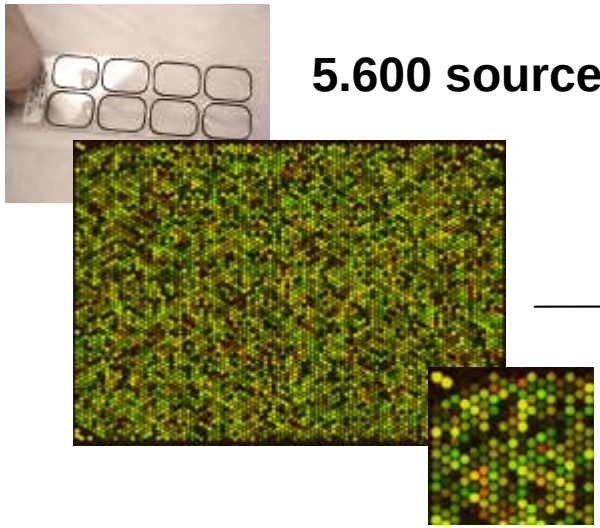
MammaPrint microarray IVDMIA



5.600 sources

XPrint Software Algorithm

Risk Assessment (High Risk or Low Risk)

232 reporter genes (incl. 70-gene profile) in nine-fold

465 normalization genes in three-fold

536 control genes in three-fold

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Tissue and Profile Discovery

Why is tissue handling today important for profile discovery tomorrow?

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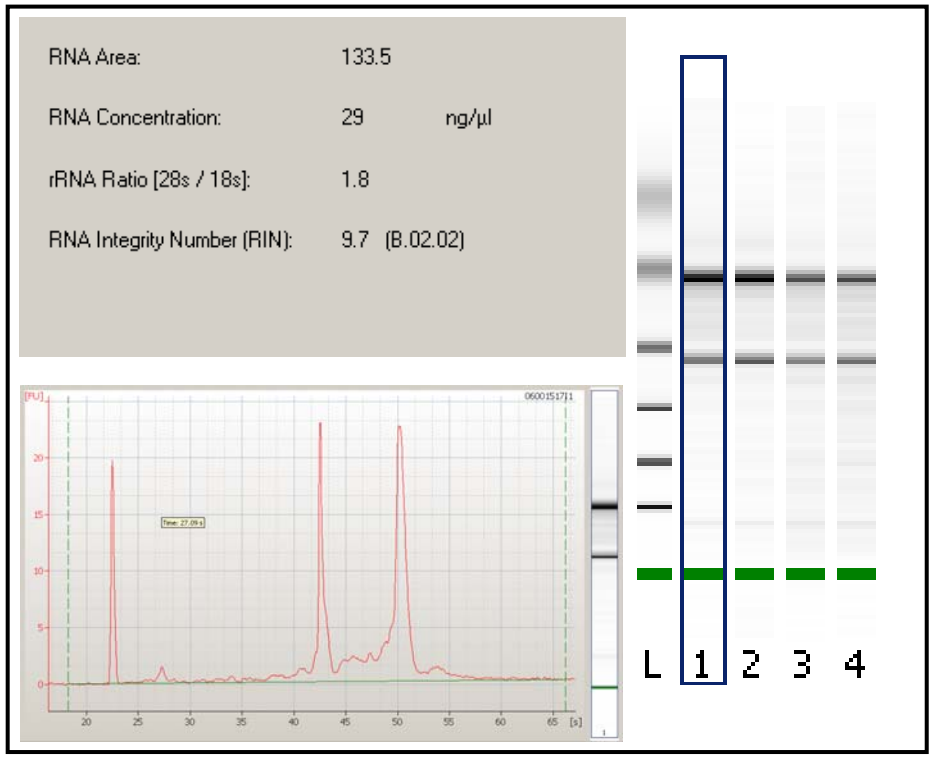
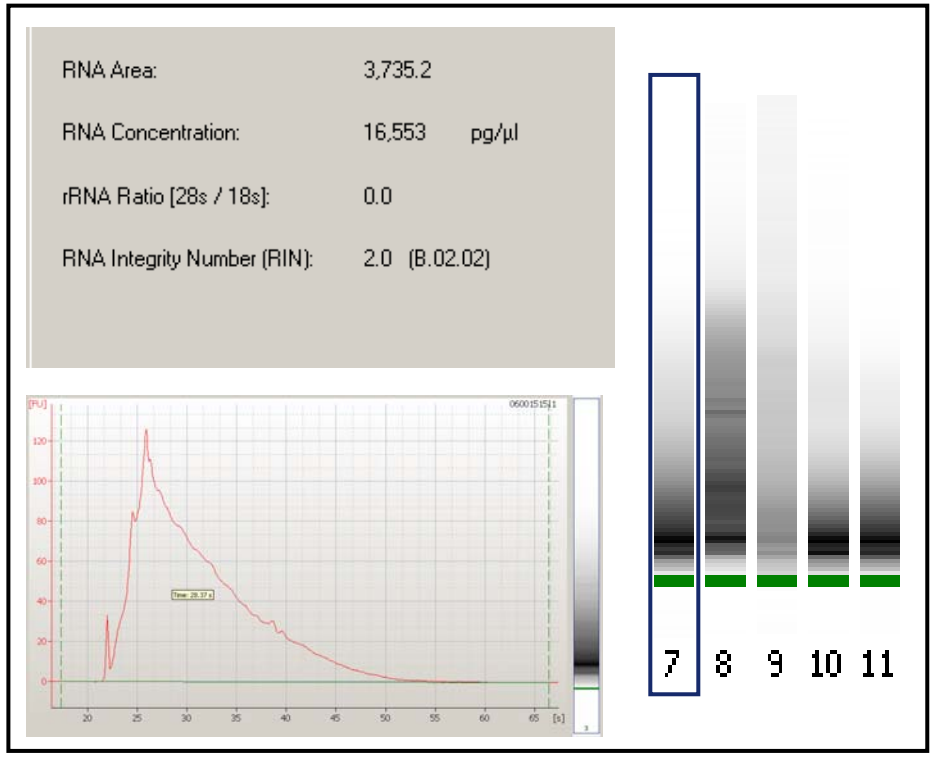
Tissue handling effects information quality!





- RNA from fresh tissue: **INTACT**
- RNA from FFPE tissue: **DEGRADED**

Conventional sampling techniques – not the optimal for profile discovery

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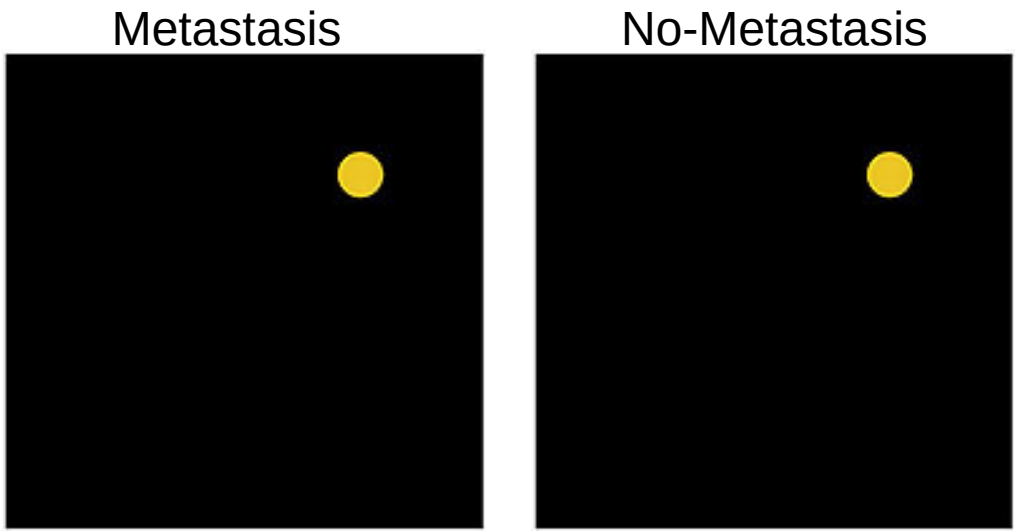
<u>Low quality</u> samples used in discovery result in; fewer genes, little information, sub-optimal profiles



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<u>High quality</u> samples used in discovery result in;
more genes, complete information, optimal profiles

Metastasis

No-Metastasis

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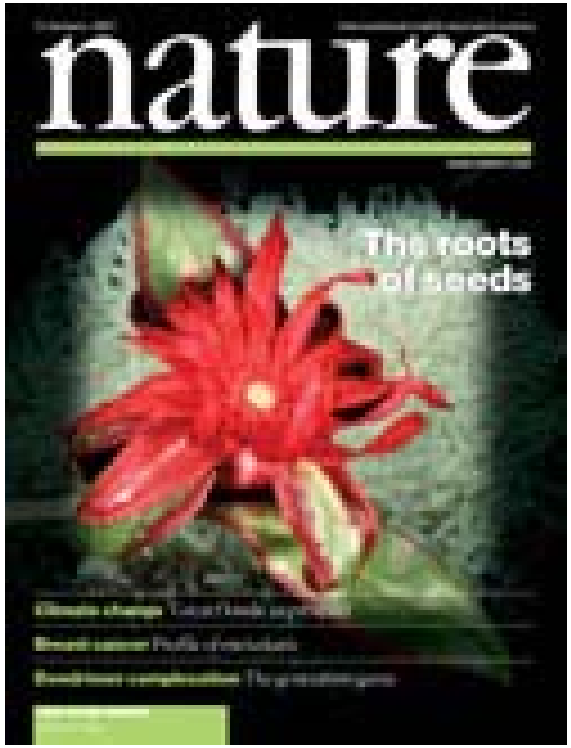
Discovery of the MammaPrint 70-gene profile



NKI-AVL
The Netherlands Cancer Institute
Antoni van Leeuwenhoek Hospital

Van ‘t Veer et al. (2002)
Nature **415**, 530-536.

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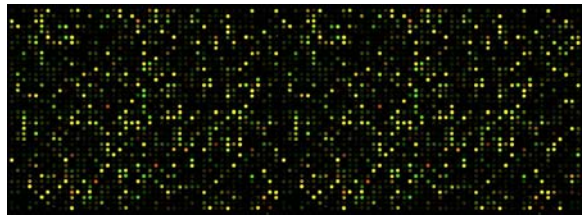
Discovery of the MammaPrint 70-gene profile

- 78 frozen breast tumors (‘83-’94)
- No adjuvant therapy
- Full clinical annotation
- Long follow-up data



Unbiased full genome
gene expression analysis

Prognosis Reporter Genes

distant metastases
< 5 years (n=34)

no distant metastases
in at least 5 years (n=44)

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Conclusion on multiplex profile discovery

In order to be able to discover high quality multiplex profiles in future, we need to start today handling patient samples in a standardized way which preserves all genetic information in the best possible way.

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Tissue handling and profile diagnostics

Why is tissue handling today important for diagnostics tomorrow?

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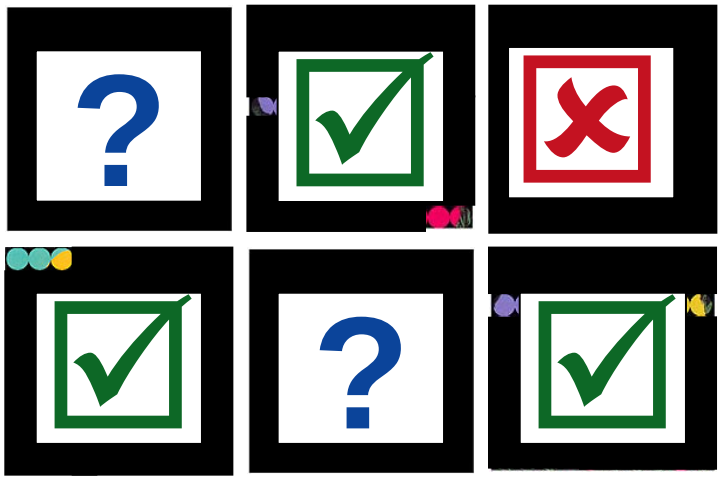
Using High versus Low Quality Samples in Diagnostics

Metastasis

No-Metastasis

High Quality Profile



High Quality Diagnostic Samples

Low Quality Diagnostic Samples

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Conclusion on Multiplex Profile Diagnostics

In order to be able to analyze patient samples on high quality multiplex profiles, which are available now or will be discovered in future, we need to start handling patient samples in a standardized way that preserves all genetic information in the best possible way.

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FDA Clearance of MammaPrint

- February 6, 2007 – Agendia acquires first FDA clearance for MammaPrint for use with *fresh frozen* tissue
- June 22, 2007 – Agendia acquires second FDA clearance for MammaPrint for use with *fresh* tissue preserved using RNARetain preservation method

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RNARetain preservation solution

- RNARetain (a.k.a. RNALater) is a commercially available, non-toxic high salt solution which is designed for diagnostic use, to preserve tissue at ambient temperature for several days without degrading RNA.
- Tissue shipped in RNARetain is considered non-infectious, non-dangerous by IATA air transport regulations, as RNARetain has bacteriostatic and virostatic capabilities.
- RNARetain is manufactured by Asuragen U.S. in compliance with the strict FDA GMP/QSR regulations.

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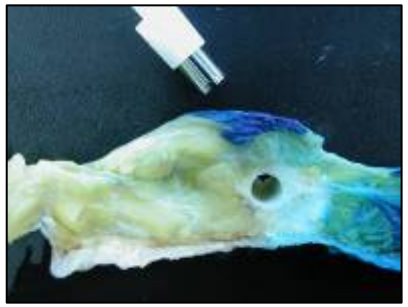
FDA cleared MammaPrint RNARetain procedure

- Take sub sample with 3mm biopsy punch
- Place sub sample in RNARetain sample container
- Ship sample using provided courier materials at ambient temperature (no cooling or freezing required)
- Sample is well preserved for at least 7 days at ambient temperature
- Upon arrival at Agendia sample is taken out of RNARetain, snap frozen in liquid nitrogen and stored at -70C



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Data evaluated for FDA clearance

- Clinical Validation
  - Clinical Claim (Intended Use)
    - Instruction for Use
    - Indication for Use
- Technical Validation (in compliance with NCCLS EP5-2A document)
  - Precision
  - Reproducibility
  - Repeatability
  - Accuracy
  - Sensitivity
  - Robustness

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Technical validation

In order to show the effect of biospecimen handling on MammaPrint, Agendia performed extensive validation studies.

Details can be found in the FDA “decision summaries” available at http://www.fda.gov (search on MammaPrint)

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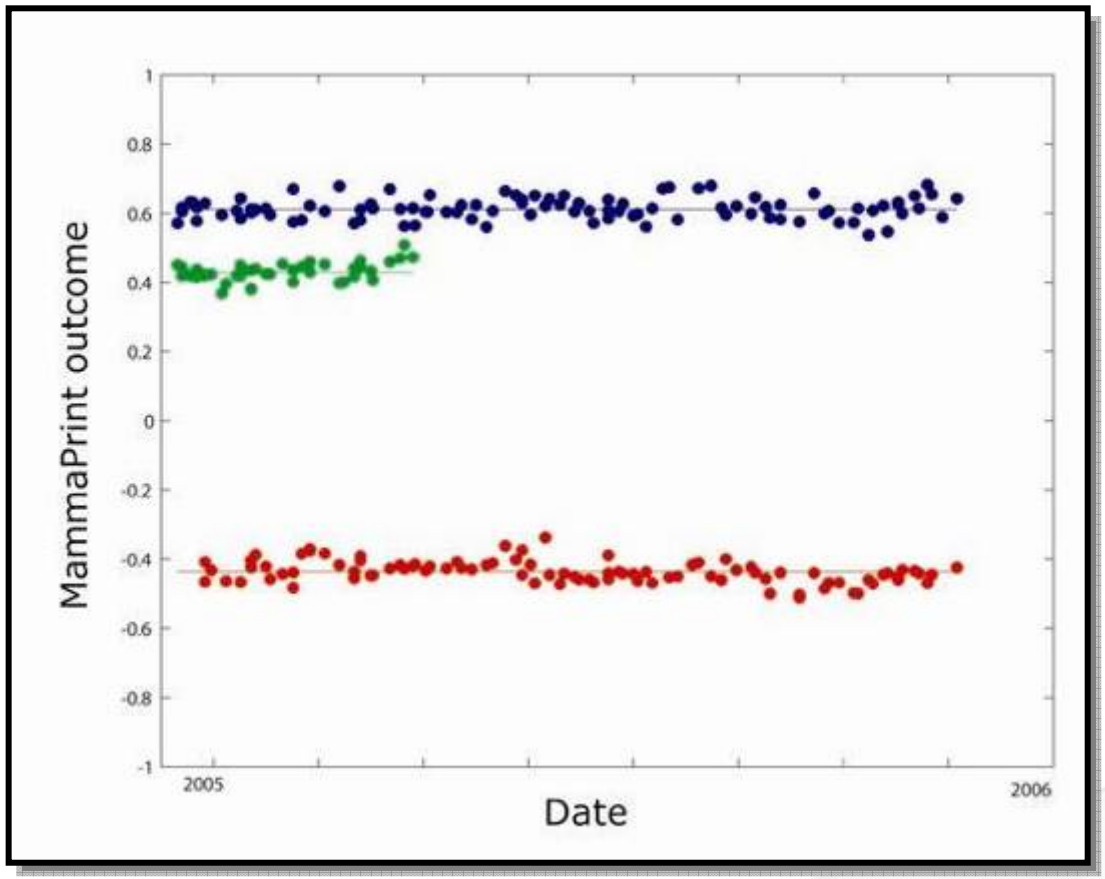
Reproducibility Starting from RNA pool

- Three different RNA controls were analyzed 254 times over a period of 1 year.
- On a MammaPrint Index scale of 2.0 the highest Stdev is 0.03.



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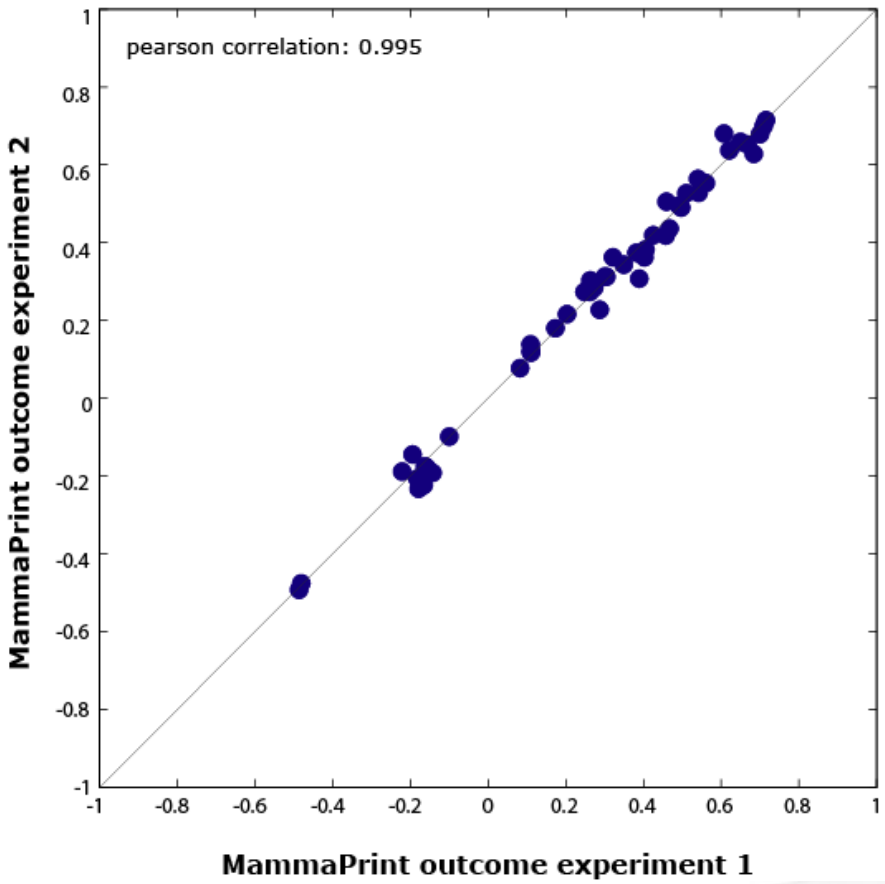
Repeatability starting from Patients

- 46 different patient samples were re-analyzed a second time starting from tissue.
- Result was a Intra Class Correlation Coefficient of .9953



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Repeatability Starting from one Labeled RNA sample

- Hybridize 1 sample on 8 different arrays on 8 different days
- On a MammaPrint Index scale of 2.0 the Stdev is 0.02.

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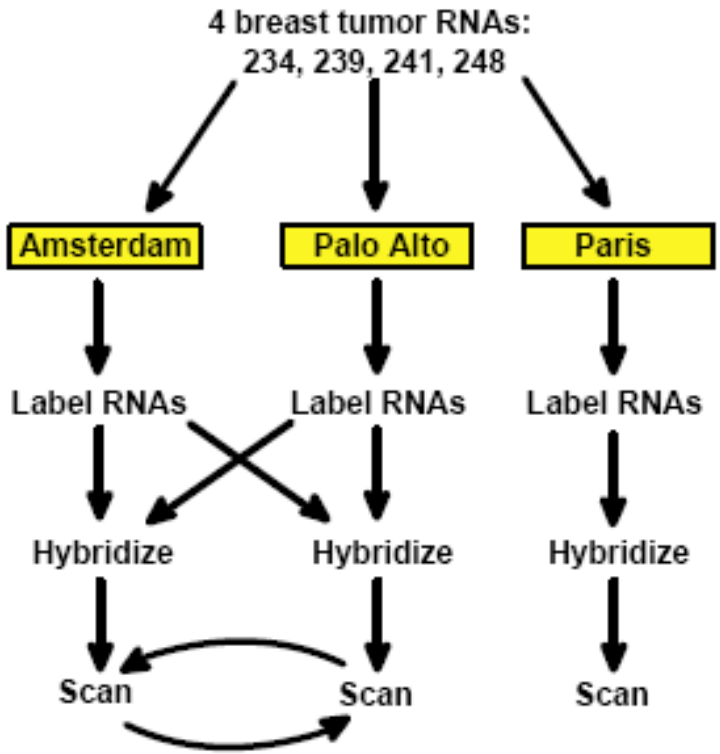
Interlaboratory comparison

- 4 breast tumor RNA’s were distributed between 3 laboratories.
- Highly reproducible results between laboratories.



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Reproducibility of Multiple Isolations starting from Tissue sample

- Duplicate Isolations of previously analyzed Tissue samples.
- No statistical significant difference in MammaPrint outcome (High Risk, Low Risk) or MammaPrint Index (on scale of 2.0) between duplicate RNA isolations.

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Detection Limit

- Validation on extensive number of separate biospecimens of same patients has shown tumor load of 30% is sufficient to produce accurate results.

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Specificity - Interference

- RNA specifications provided to FDA were adequate to exclude the presence of any effect of likely interfering substances in the biospecimen (e.g. necrotic tissue, stromal tissue, blood, normal tissue)

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RNARetain versus Frozen comparison

- Comparison of 50 paired patient tissue samples and 6 mice xenografts preserved two ways. Fresh Frozen and RNAretain ambient temperature.
- Methods show identical analytical results (MammaPrint Index) and Prognostic outcome (High Risk/Low Risk).

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Influence of Adverse Weather conditions on RNARetain preservation – Historical data

- Homogeneous group of patient samples from a clinical trail were selected over a time period of a year (n=204).
- Influence of Air temperature was investigated on High Risk/Low Risk balance and RNA quality rejection rate.
- Temperature does not influence rejection rate or High Risk / Low Risk balance.

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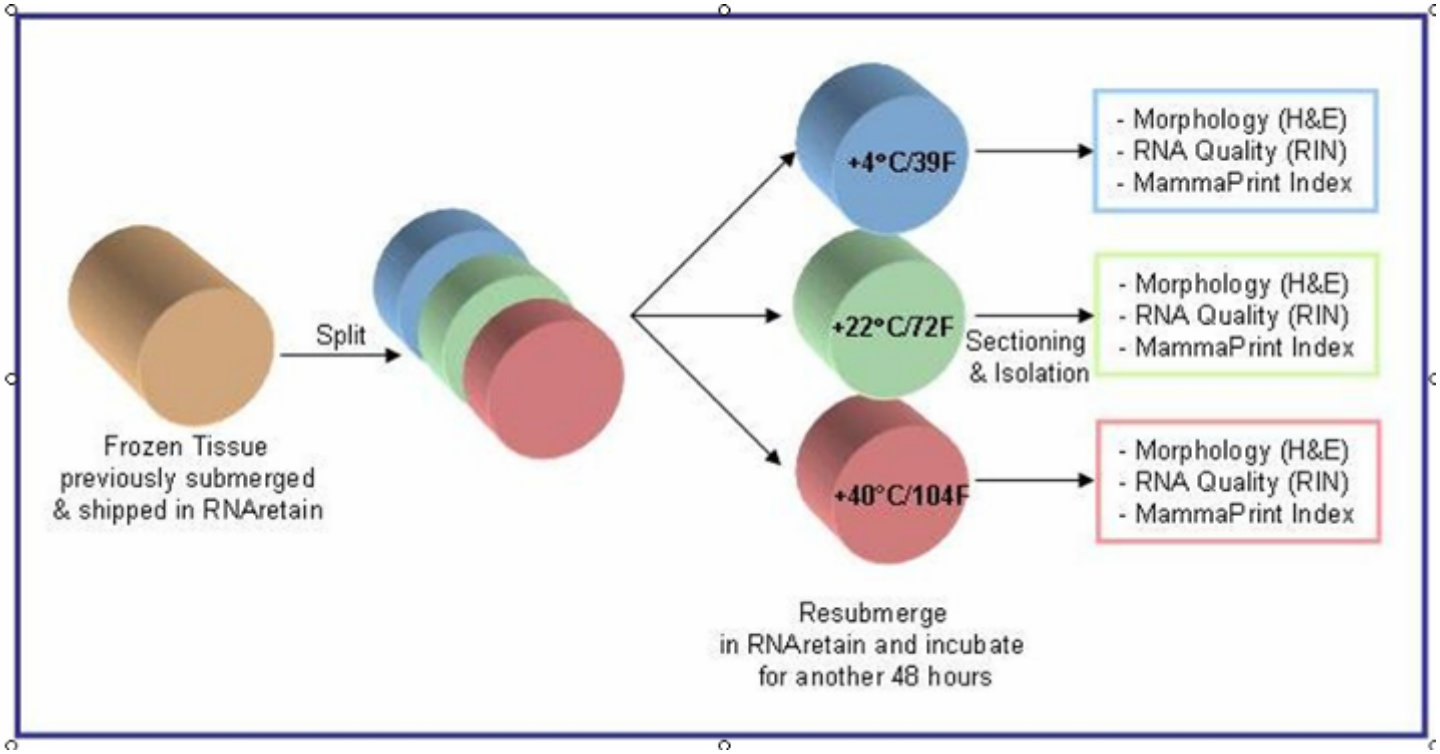
Influence of Adverse Weather conditions on RNARetain preservation – extreme system stress



- Adverse Weather Conditions did not negatively influence morphology, RNA quality and MammaPrint Index

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Conclusion

The RNARetain preservation method of fresh tissue has been proven to be robust, precise, reproducible, repeatable and accurate

It’s convenient, non-toxic, cheap, fast and readily available

The procedure is highly suitable for multiplex discovery and multiplex diagnostics

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Summary

- High quality tissue samples give more informative multiplex profiles and ensure more accurate diagnostic results
- The MammaPrint RNARetain procedure is highly suitable for multiplex discovery and multiplex diagnostics

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Thank You!

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