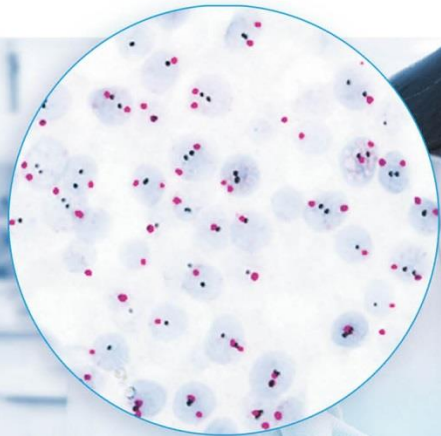


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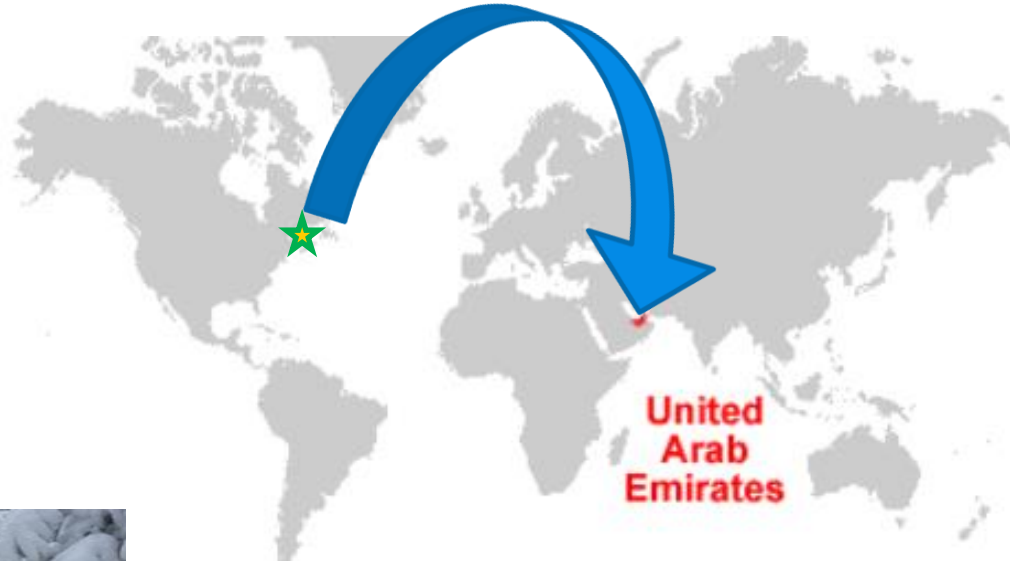
# ISH & It's Colorfull Stories

*Best practices & guidance explained*



## Ossayed (Oss.) Al Awor

- *Senior Application Specialist, Roche Molecular Solution, Roche Diagnostics Middle East, Dubai, UAE*



# ....in his spare time

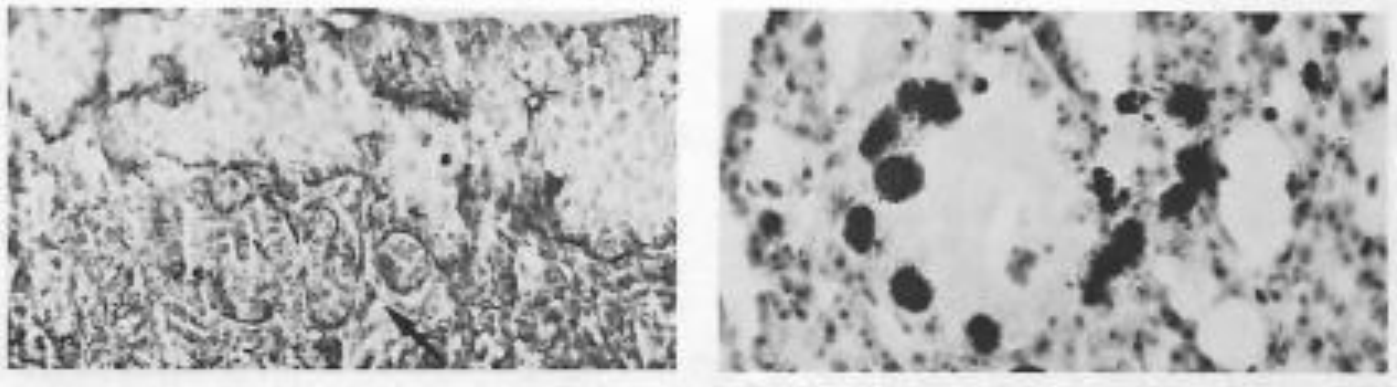


# Objectives

- Different ISH techniques
- Create awareness about the impact of Pre Analytics in **ISH**
  - Poor and Best Practice through published references
  - Guidelines
- Roche implication to improve patient management.

# History

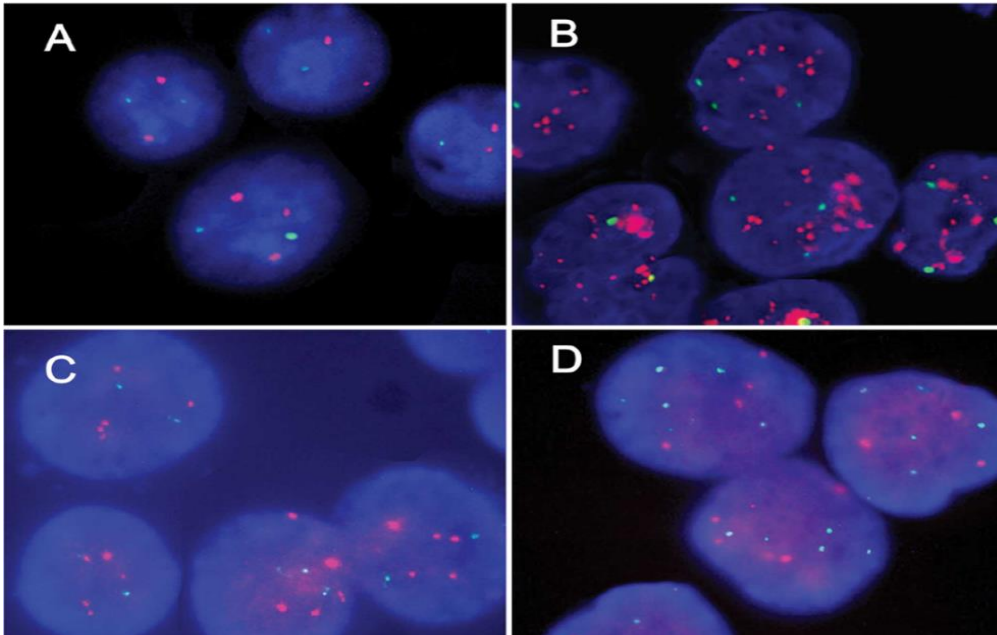
- Mary-Lou Pardue and Joseph G. Gall 1968



- probe was labeled with  $^3\text{H}$  and the hybrid was detected by autoradiography

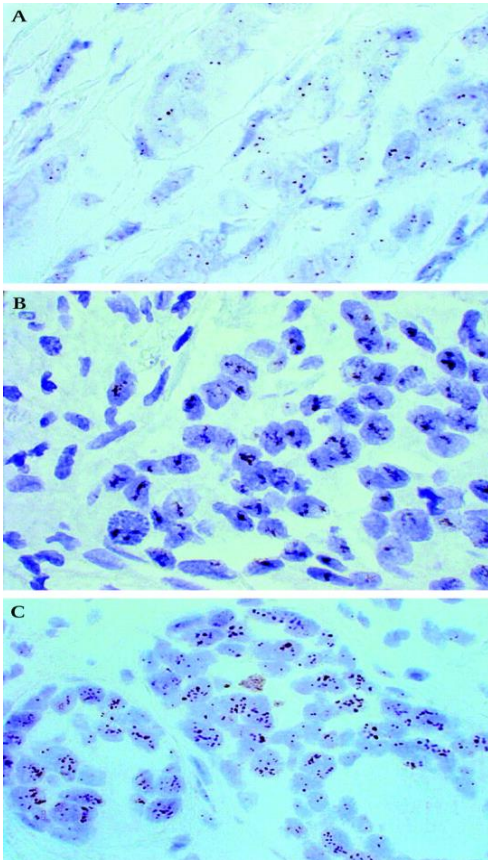
# History

- **Examples of HER2 detection using the Florescence in situ hybridisation (FISH) technique in breast carcinoma.**



# History

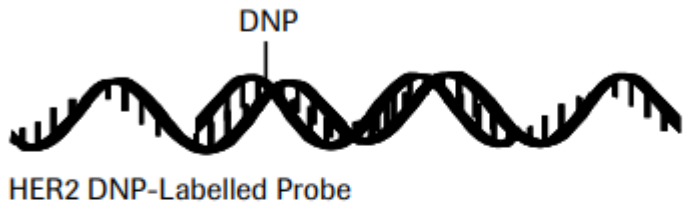
- **Examples of HER2 detection using the chromogenic in situ hybridisation technique in breast carcinoma.**



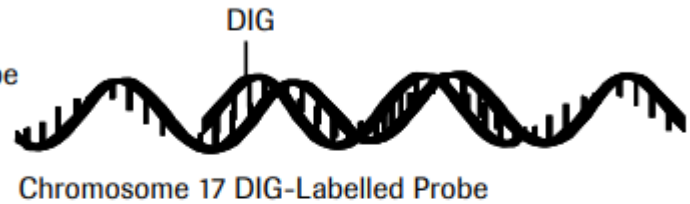
# DDISH Principle

Figure 1.

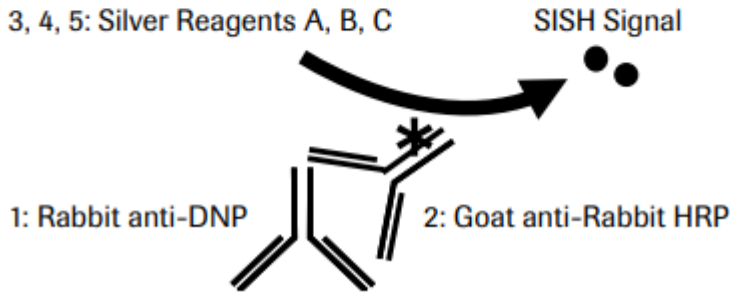
**A. *ultraView* SISH DNP**



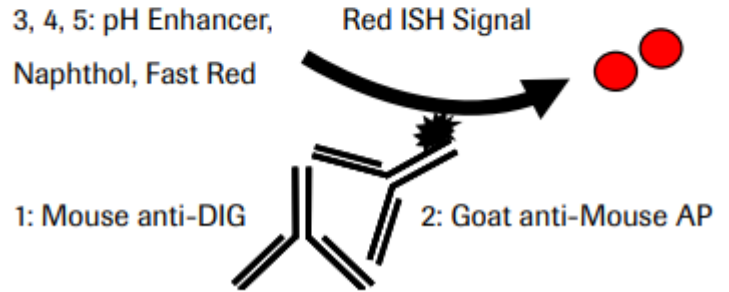
Cocktailed  
HER2 and Chr17 Probe



**B. *ultraView* SISH DNP Detection Kit**



**C. *ultraView* Red ISH DIG Detection Kit**



## Automated ISH testing differs from IHC

- Molecular assays, like *in-situ* hybridization (ISH), are complex processes and are more sensitive to pre-analytic variability and contamination than IHC
- Fully automated ISH requires careful and consistent instrument maintenance and decontamination according to the recommended schedules
- Following recommended guidance on slide handling, protocol optimization and reagent use can help maintain consistent performance on all automated assays

# Tissue Guidance

- Assay recommendations:

- **Fixation**

- Tissue fixed in 10% neutral buffered formalin (NBF)
    - Tissue fixed for 6-48 hours<sup>1,3</sup> (Core needle biopsies may require shorter fixation time)
    - <1 hour ischemia time<sup>1,3</sup>

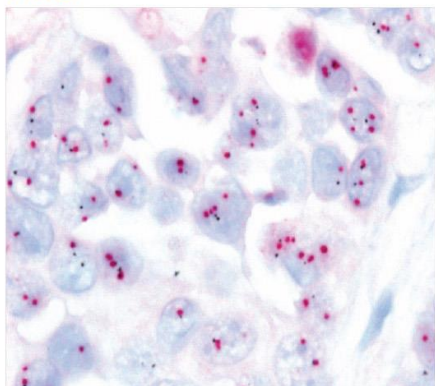


- **Tissue**

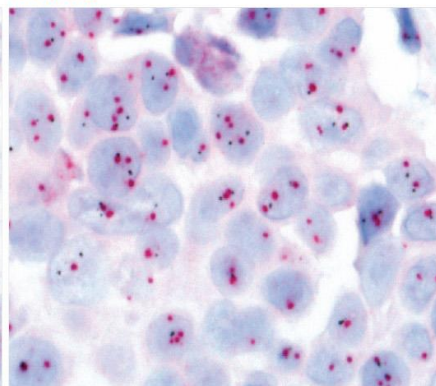
- Paraffin embedded
    - Sectioned at approximately 4 microns
- A recent ISH study showed that strict implementation of fixation procedures resulted in a 64% reduction in “inconclusive” cases.<sup>2</sup>

# Tissue Fixation

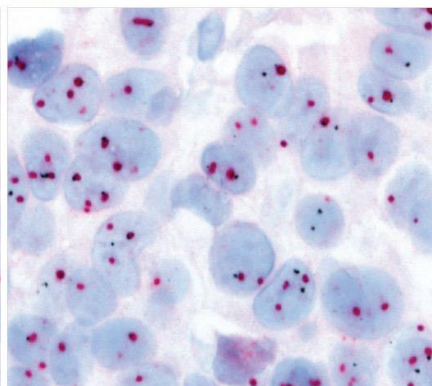
1 Hour



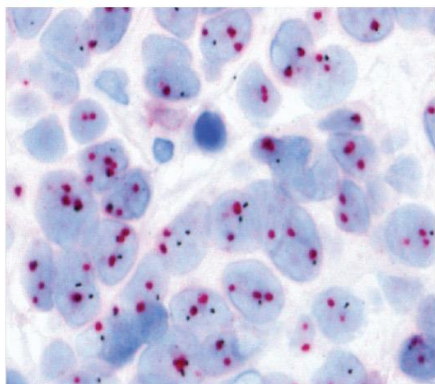
3 Hours



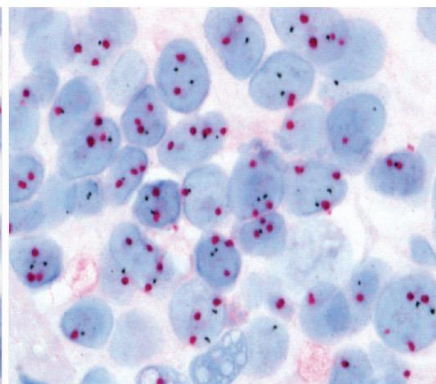
6 Hours



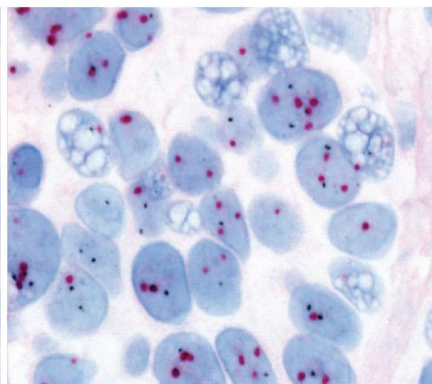
12 Hours



24 Hours



48 Hours



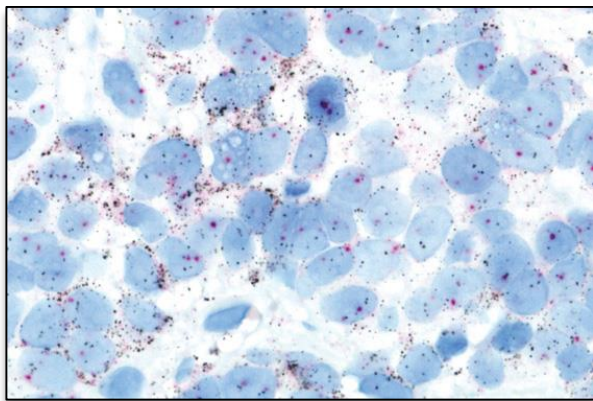
MCF7 xenograft core, 10% NBF, 60X.

**Under fixed 10% NBF tissue will not exhibit optimal staining and will show poor, “over-digested” morphology. Lighter counterstain is evident at short fixation times.**

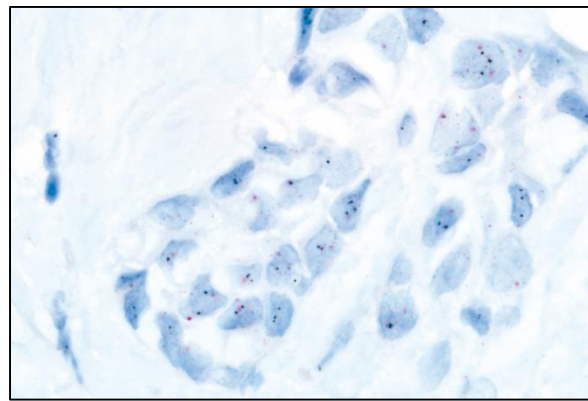
**Recommended fixation for INFORM HER2 Dual ISH is 6-48 hours<sup>1,3</sup>**

# Water quality matters

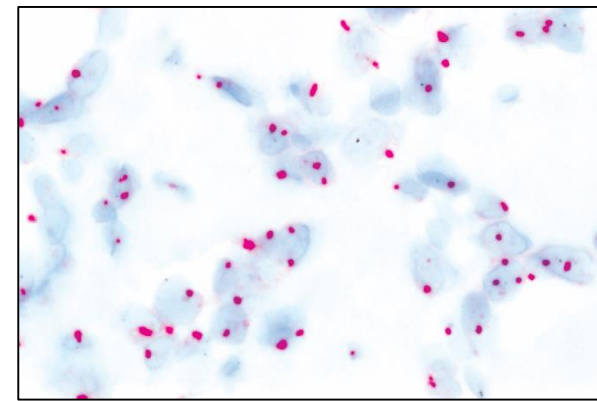
- Ensure the best possible water quality is used in the instrument<sup>3</sup>
  - Bulk reagents mixing require clean deionized or distilled water
  - Contamination can be introduced into the instrument or bulk fluids via poor water quality
- Contamination can result in increased failures due to speckling, weak staining or poor morphology\*



HER2 and Chr17, 60x, inadequate due to SISH speckling.



HER2 and Chr17, 60X, inadequate due to weak/absent Red ISH staining in positive control nuclei and tumor nuclei.

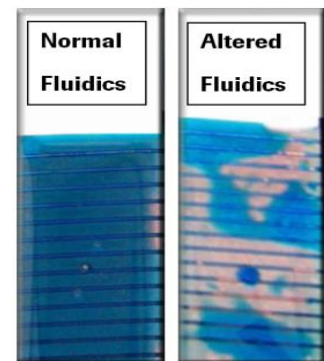


HER2 and Chr17, 60X, inadequate due to lack of SISH staining in positive control nuclei and tumor nuclei.

\*Internal Product Technical Support data on instrument decontamination procedures on ISH assays

# Microscope Slides

- Use Superfrost Plus microscope slides or equivalent positively charged slides<sup>5</sup>
  - **Limit exposure to heat and humidity**
    - If storage is necessary, desiccate slides
  - **Minimize time slides are stored outside of packaging**
    - Place fresh cut tissue on new + charged slides to ensure fluidics
  - **Minimize plastic exposure**
    - Remove all sources of plastic in ovens
    - Use glass or metal containers and racks
  
- Submerge glass in water only once when collecting tissue<sup>4</sup>
  - No “double-dipping”



# Barcode label placement is extremely important

*Misplaced labels can cause speckling/slide drying*

- Labels must be centered and applied to the slide without any overhang

## No double labels or re-application of labels



Default/Optimal

1/2mm shift

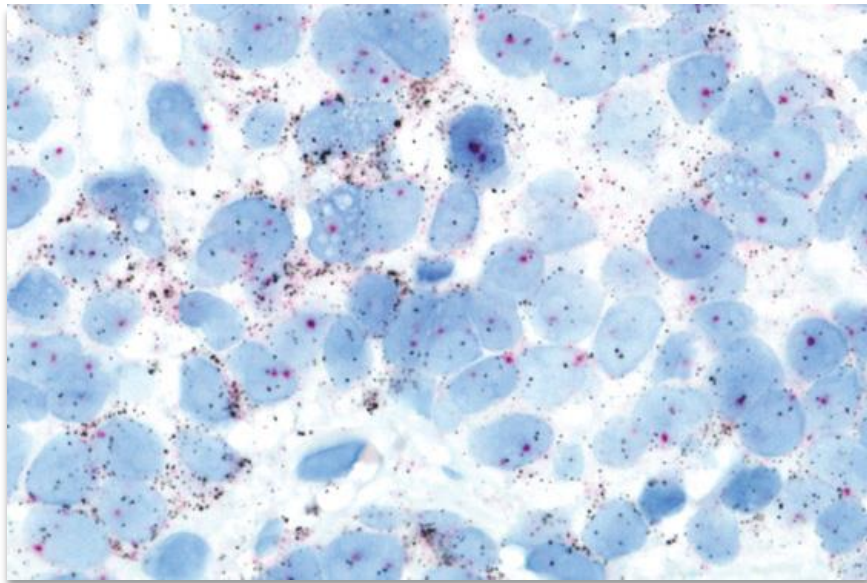
1mm shift



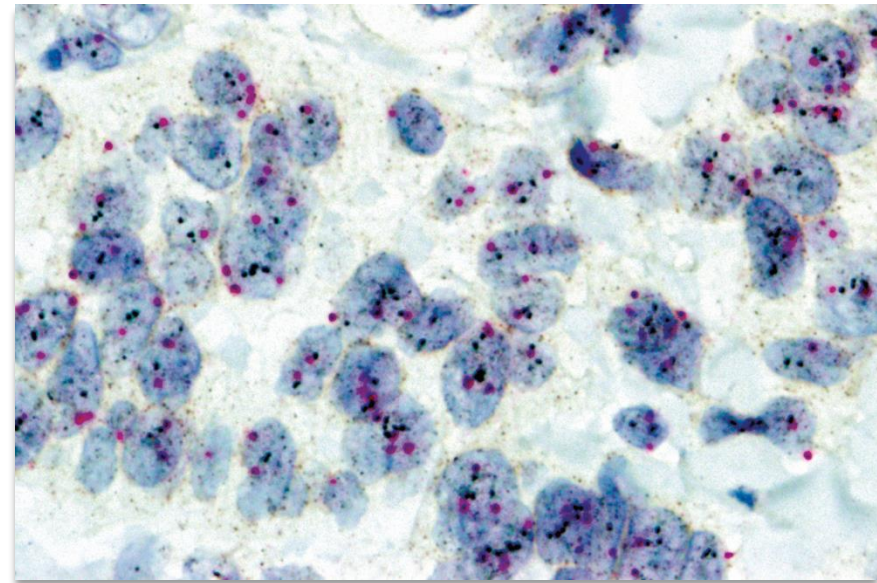
Misplaced labels may create a channel or ramping area where reagents may wick or be diverted off the slide!

## Poor barcode label placement

If fluid levels are not maintained on the slide during hybridization, speckling and slide drying are likely to occur<sup>4</sup>



HER2 and Chr17, 60x SISH speckling

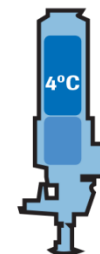


HER2 and Chr17, 60x SISH dust, slide drying

# Pre-run processing

## Before every run:

- Inspect all tips and remove plugs or residual reagent with a non-metallic tool and prime meniscus to end of dispenser
- Store dispensers capped at 4° C when not in use

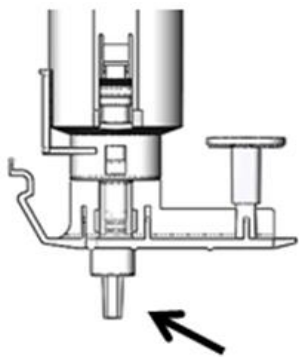


1. INSPECT  
NOZZLE TIP

2. INSERT PIPETTE TIP  
HALF-WAY UP  
NOZZLE TIP

3. MOVE PIPETTE TIP  
TO INSIDE SURFACE  
TO COLLECT RESIDUE

4. RESIDUE  
REMOVED



# Pre-run processing

## **Your Roche application specialist will help you optimize your staining protocol at installation**

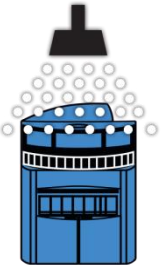
- 1-2 additional rescue protocols may also be created to help address differences in pre-analytics

## **Priming the instrument prior to each run can help ensure greater staining consistency\***

- Prime the instrument at least once before each HER2 Dual ISH run
- If the instrument has been idle for more than 48 hours, multiple instrument primes may be necessary before performing a HER2 Dual ISH run

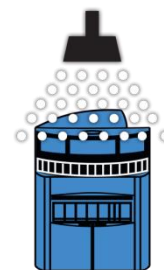
# Post-run processing

- Avoid alcohol baths, acetone and extended xylene incubations during the post-run dehydration process<sup>4</sup>
  - Alcohol baths, acetone and extended xylene incubations will dissolve Red ISH Chromosome 17 signals
  - Do not use the following mounting media: Eukitt (EMS) , Entellen New, Entellen (Merck), HSR (Sysmex) and Malinol (Muto Chemical).
  - These mounting media are not compatible with HER2 Dual ISH, and lead to weak or faded HER2 SISH signals<sup>4</sup>

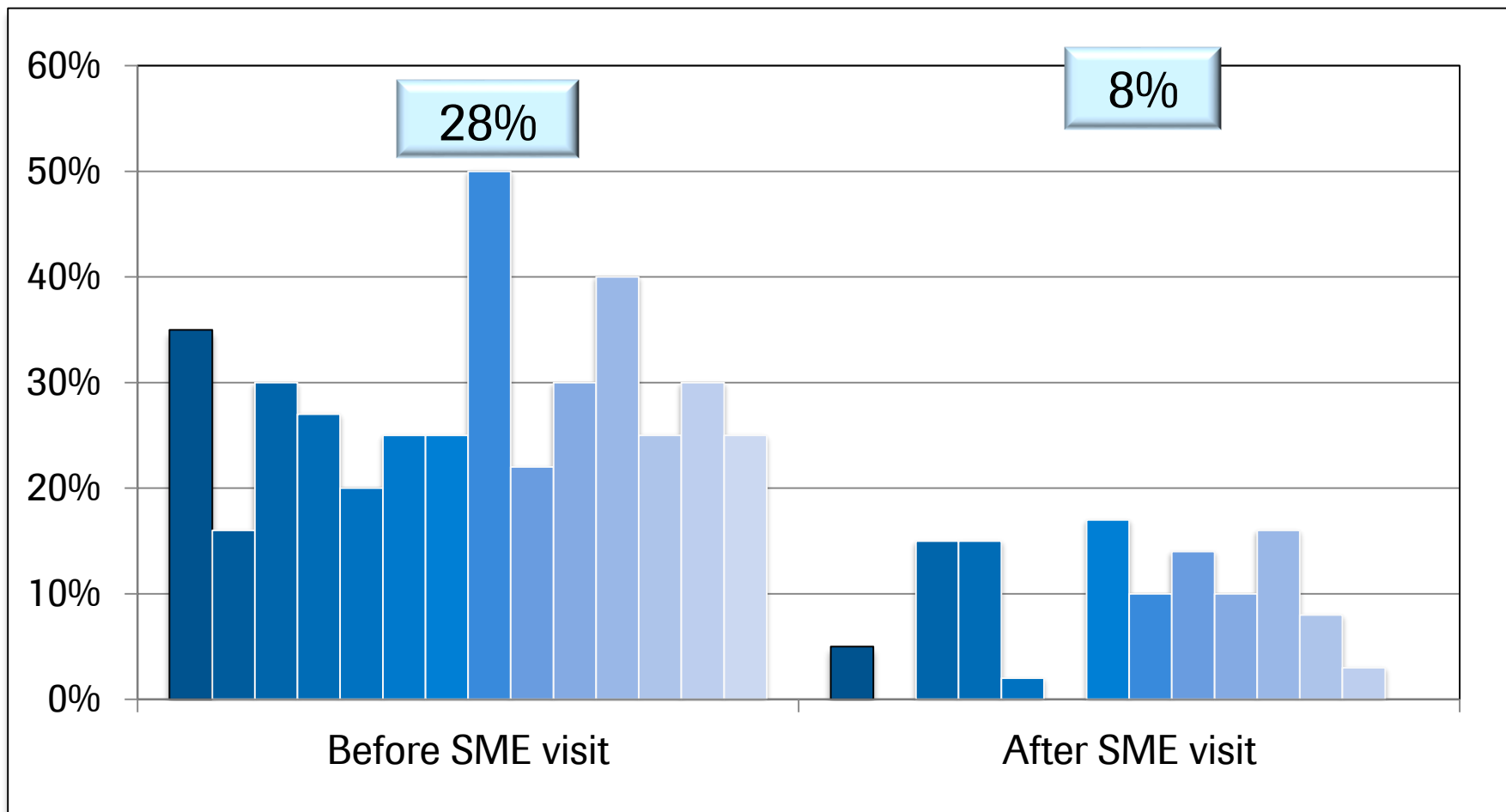


# Instrument maintenance and decontamination

- Decontaminate instrument quarterly per operator's manual
  - Ensure decontamination and rinsing of instrument bottles and bulk fluid carboys
  - Ensure sufficient flushing of instrument following decontamination procedure to remove any residual decontamination reagent
- Ensure that Level 1/Preventative maintenance procedures are performed according to recommended schedule



# HER2 Dual ISH repeat rates at sites seeking help



\*Internal data Roche subject matter experts (SME) provided laboratory sites with guidance on ISH best practices, Jun-Dec 2015

# Roche: what makes us different

*Optimally positioned for the future*



**Leader in Diagnostics**



**Leader in Pharma**

**We combine expertise in molecular biology and diagnostic technologies to benefit patients**



**Early detection**

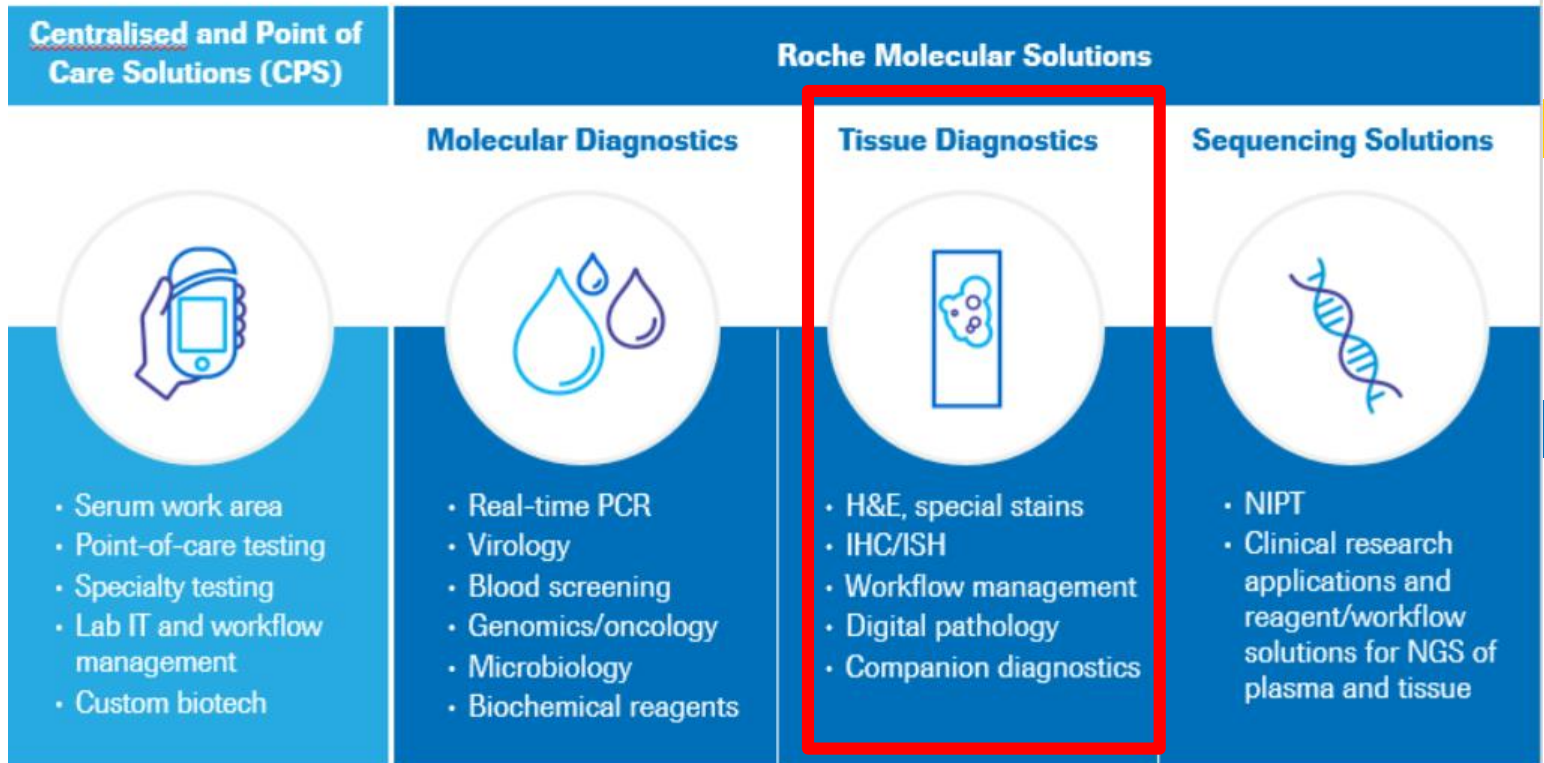
**Prevention**

**Diagnosis**

**Therapy**

**Therapy monitoring**

# Roche Diagnostics



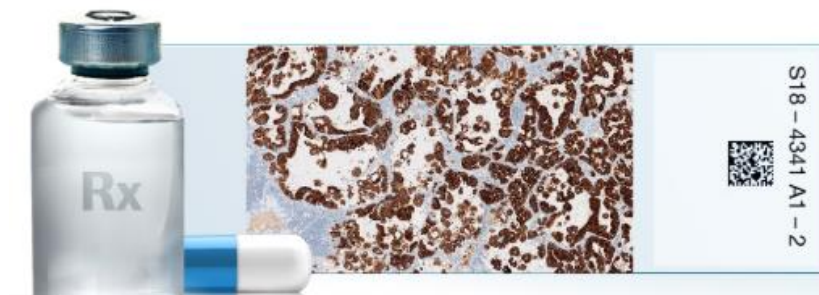
Switzerland

Pleasanton, USA

Tucson, USA  
(VENTANA)

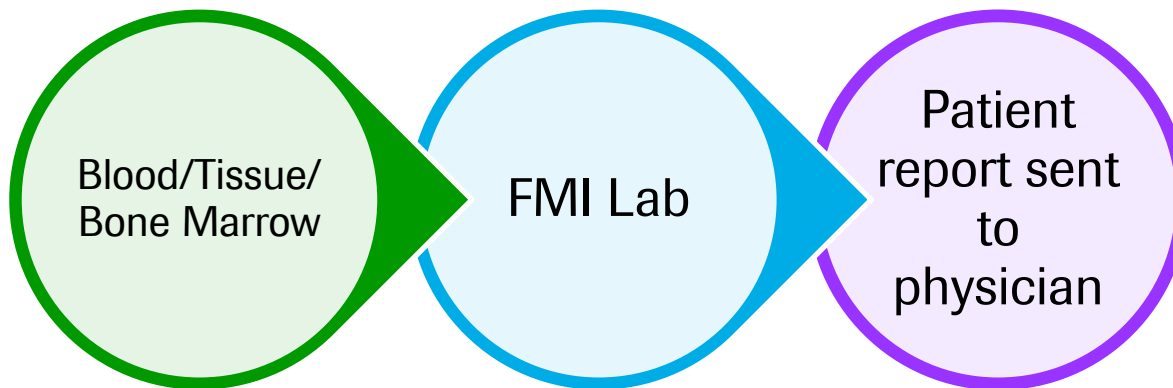
Pleasanton, USA

# Roche Pharma *Switzerland*



# Foundation Medicine

- Headquarters Cambridge, USA
- Genomic profiling assays (NGS) for cancers
- Molecular profile for targ



**BIOMARKER FINDINGS**

**Tumor Mutational Burden - TMB-Intermediate (6 Muts/Mb)**

3

10 Trials *see p. 19*

**Microsatellite status - MS-Stable**

**GENOMIC FINDINGS**

**EGFR - L858R**

3

10 Trials *see p. 22*

**PIK3CA - E545K - subclonal**

**THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)**

- Atezolizumab
- Durvalumab
- Nivolumab
- Pembrolizumab

No therapies or clinical trials. *see Biomarker Findings section*

**THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)**

- Afatinib
- Dacomitinib
- Erlotinib
- Gefitinib
- Osimertinib

none

2

**THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)**

- Avelumab
- Cemiplimab-rwlc

2

**THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)**

- Cetuximab
- Lapatinib
- Panitumumab

Everolimus

# FoundationONE report



BIOMARKER FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Tumor Mutational Burden - TMB-Intermediate (6 Muts/Mb)	Atezolizumab Durvalumab Nivolumab Pembrolizumab	Avelumab Cemiplimab-rwlc
10 Trials see p.19		
Microsatellite status - MS-Stable	No therapies or clinical trials. see Biomarker Findings section	
GENOMIC FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
EGFR - L858R	Afatinib Dacomitinib Erlotinib Gefitinib Osimertinib	Cetuximab Lapatinib Panitumumab
10 Trials see p.22		
PIK3CA - E545K - subclonal	none	Everolimus

# Histology report

**PATIENT NAME**  
Date of Birth  
Medical Record Number

**PHYSICIAN NAME**  
Contact information

**SPECIMEN CASE NUMBER**  
Date specimen obtained  
Date specimen processed in lab

**CLINICAL HISTORY:** 55 year-old female with a palpable breast mass and suspicious calcifications on mammogram

**BODY SITE AND PROCEDURE:** Breast, left at 12:00, 3 cm from nipple (ultrasound-guided core needle biopsy)

**DIAGNOSIS:** Infiltrating ductal carcinoma, Nottingham histologic grade 2 of 3. SEE NOTE.  
**NOTE:** The carcinoma is positive for ER (100%, Strong) and PR (90%, strong), and is negative for HER-2 (IHC Score 0).

**GROSS DESCRIPTION:** Specimen is received in formalin, labeled with the patient's name, and consists of 3 cores of tan tissue measuring 0.2 cm in width and 0.9-1.3 cm in length. The specimen is entirely submitted in 1 block (A).

Tissue is not preserved and handled appropriately

# References

1. Babic A, et al. The impact of pre-analytical processing on staining quality for H&E, dual hapten, dual color in situ hybridization and fluorescent in situ hybridization assays. *Methods*. 2010 Dec: 52(4): 287-300. doi: 10.1016/j.ymeth.2010.08.012.Epub 2010 Aug 31.PMID: 20807574
2. Middleton LP, et al. Implementation of ASCO/CAP HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence in situ hybridization concordance and decreases the number of inconclusive cases. *Arch Pathol Lab Med*. 2009 May: 133 (5): 775-780. doi: 10.1042/1543-2165-133.5.775
3. Package Insert-INFORM HER2 Dual ISH DNA Probe Cocktail Ventana.com web portal: <http://productlibrary.ventana.com/> (Search product 800-4422 or 780-4422 US only)
4. Interpretation Guide-Ventana INFORM HER2 Dual ISH DNA Probe Cocktail Assay Ventana website: <http://productlibrary.ventana.com/> (Search product 800-4422 or 780-4422 in the US only)
5. Recommended Slide Storage and Handling, Ventana website: [http://productlibrary.ventanamed.com/ventana\\_portal/documents/1011752ENb.pdf](http://productlibrary.ventanamed.com/ventana_portal/documents/1011752ENb.pdf)

*Doing now what patients need next*