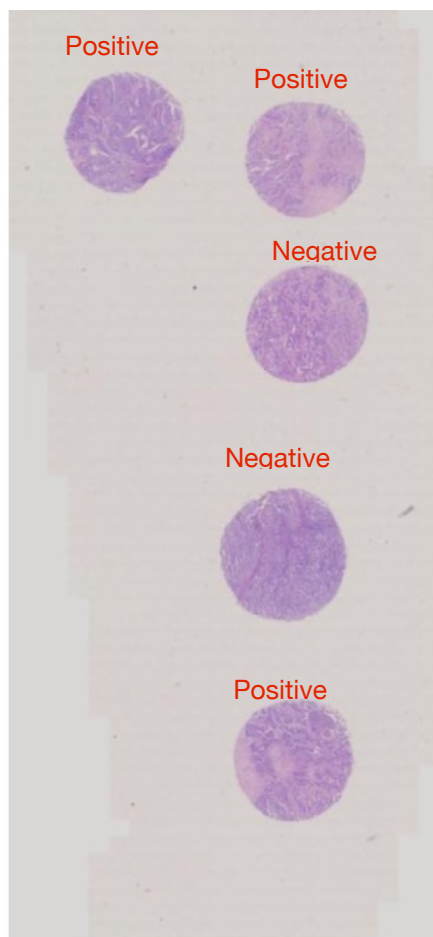
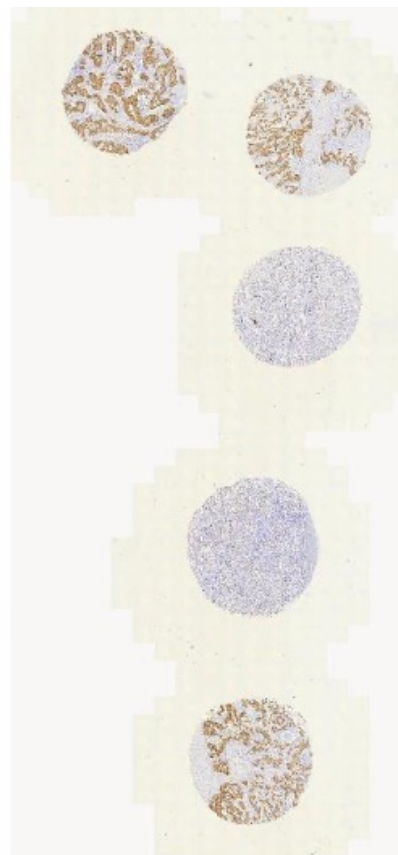


## Her2



HE staining in scanner view. The scores are median/average for each.



Her2 immunostaining, scanner view

### Provided sections in this run:

TISSUE	IHC	FISH
Breast carcinoma	3	Positive
Breast carcinoma	3	Positive
Breast carcinoma	0/1	Negative
Breast carcinoma	0/1	Negative
Breast carcinoma	3	Positive

\* Her2-status and staining pattern as characterised by core participating laboratories using the polyclonal antibody and Herceptest. FISH findings were derived from available sources that are different for individual cases.

\* All tissues were fixed in 10% neutral buffered formalin for 24-48 hours and processed according to Allison et al



Criteria for assessing staining as optimal included:

## 1. Staining reaction

1.1. The staining of the laboratory is assessed and scored by the laboratory as per the standard protocol of interpretation of IHC staining by ASCO/CAP guidelines. Following results are provided:

1.1.1. "Optimum"

1.1.2. "Understanding of positive case"

1.1.3. "Overstaining of negative case"

1.2. When participant has failed to stain certain section (NA), no result is provided with a comment "Missing tissue section":.

1.3. The assessor is blinded to the score done by the participant

## 2. Interpretation of stained section by the participant

2.1. The participant is requested to put the score on glass slide as well as the online protocol filling form FOR EACH SECTION.

2.2. When such score is available, it is put up in the chart and compared with the assessment done by us and reported. Following results are provided:

2.2.1. "Satisfactory"

2.2.2. "Underreporting of the staining"

2.2.3. "Over-reporting of the staining"

2.3. When such score is not available (NA), no comment is made on interpretation reflected as "Not possible as interpretation is not provided". Such laboratories are advised to put up score in next run as they would not be assessed for interpretation.

**2.4. As such, there can not be overstaining of negative case when interpretation is correct. The given comment is just to address comparison with FISH testing and not an adverse comment on staining.**

## 3. Result on cases positive by FISH

3.1. If a case is positive with FISH (Cases 1,2, and 5 in this run) AND THE PARTICIPANT HAS SCORED 3+, it is the most desirable situation. It is reported by us as "Acceptable"

3.2. If a case is positive with FISH (Cases 1,2, and 5 in this run) AND THE PARTICIPANT HAS SCORED 2+, it implies "unnecessary FISH testing but no harm to the patient" as the patient would ultimately be considered eligible for treatment with targeted therapy.

3.3. If a case is positive with FISH (Cases 1,2, and 5 in this run) AND THE PARTICIPANT HAS SCORED 1+ OR 0, it is the most undesirable situation because she will not be tested with FISH and will be deprived of potentially beneficial targeted therapy. It is reported by us as "Missing on positive case-Harmful to the patient"

4. **Implication when subsequent FISH testing is advocated:** This is extension of the point 3.2 in above paragraph. This is reported as either "Acceptable" or "Unnecessary FISH implied"

## 5. Result on cases negative by FISH

5.1. If a case is negative with FISH (Cases 3 and 4 in this run), AND THE PARTICIPANT SCORES 3+, it is the most undesirable situation as the patient may receive costly targeted therapy without any benefit from it. Reported as "Harmful to the patient"

5.2. If a case is negative with FISH (Cases 3 and 4 in this run), AND THE PARTICIPANT SCORES 2+, it will be subjected to FISH that would be financially draining but no harm would happen to the patient. Reported as "Unnecessary FISH testing-no harm to the patient"



- 5.3. If a case is negative with FISH (Cases 3 and 4 in this run), AND THE PARTICIPANT SCORES 1+ OR 0, it is the most desirable situation. Reported as "*Acceptable*"
- 5.4. "When participant fails to provide either stained section or interpretation, "*Not possible to comment*" is mentioned in the result.

Turn overleaf for Analysis



## Break up of results on staining criteria

Result	Participants
Optimum	32
Overstaining of a negative case	2
Understaining of positive case/s	2
Not available	3

## Break up of results based on technique

Result Technique	Optimum	Overstaining of a negative case	Understaining of positive case/s	Not available	Total
Manual	18	2	1	5	26
Automated Ventana Bench Mark	4			1	5
Automated Ventana Bench Mark Ultra				1	1
Automated Ventana Bench Mark XT	4		1		5
Automated, Ventana Benchmark GX	1			1	2

## Clones used in the run by participants

Clone	Participants
4B5	12
CB11	1
CerbB-2	5
EP3	9
QR003	3
RABBIT MONOCLONAL ANTIBODY(B2-EP3)	1
RMPD)08	1
SP3	3



## Break up of results based on the clones used

Clone	Result	Optimum	Overstaining of a negative case	Understaining of positive case/s	Not available	Total
4B5		8		1	3	12
CB11		1				1
CerbB-2		3	1	1		5
EP3		9				9
QR003		2			1	3
RABBIT MONOCLONAL(B 2-EP3)		1				1
RMPD)08		1				1
SP3		2	1			3

## Break up of results based on the vendors

Vendor	Result	Optimum	Overstaining of a negative case	Understaining of positive case/s	Not available	Total
Biocare		2	1			3
BioGenex		3				3
Dako		4		1		5
Diagnostic Biosystems		1				1
MASTER DIAGNOSTICA			1			1
Path n situ		6				6
Quartett		2				2
Quartett, P-H001-70					1	1
Thermo		1				1
Ventana		8		1		9
(blank)		5				5



## Break up of results based on format

Format	Result	Optimum	Overstaining of a negative case	Understaining of positive case/s	Not available	Total
RTU		22	2	1	1	29
Concentrated.		5		1		6
Blank		5			4	9

## Break up of results based on temperature of dewaxing

Dewax Minutes	Result	Optimum	Overstaining of a negative case	Understaining of positive case/s	Not available	Total
37			1			1
>75		1			1	2
55-59		1	1			2
60-65		12		1		13
66-70		4				4
71-75		7			1	8
RT		1				1
(blank)		6		1	5	12

## Break up of results based on pH of retrieval buffer

pH	Result	Optimum	Overstaining of a negative case	Understaining of positive case/s	Not available	Total
6.0-6.9		4	1			5
7.0-8.0		3				3
8.1-9.0		20	1	1		22
NA		6		1	3	10



### Protocol used by the top 3 in this run

VARIABLE	1	2	3
Technique	Manual	Manual	Automated Ventana Bench Mark XT
Clone	EP3	QR003	4B5
Vendor	BioGenex	Quartett	Ventana
Format	RTU	RTU	RTU
Batch/Year	AN7260222A	414402, NA	H35077; 2022
Expiry	February 2024	2024-01	10/10/2023
Dewaxing temperature	62-68 degree celsius	65°C	room temp in milk
Retrieval	HIER	HIER	HIER
Enzyme	NA	NA	Not applicable
HIER	Microwave Oven	Microwave oven	Ventana CC1 mild
Peak T and Time	At 900 Watt-20 minutes At 540 Watt-20 minutes Temperature: 95 degree celsius	110°C, 15 mins (5+5+5)	As per Ventana automated protocol
Peak Pressure and Time	NA	NA	As per Ventana automated protocol
Retrieval Buffer	In-House	Company provided, EnVision FLEX TARGET RETRIVAL SOLUTION	Ventana CC1 mild
pH	pH-8.0	9.0	8.4
Blocking	3%H <sub>2</sub> O <sub>2</sub> for 10 minutes	EnVision FLEX PEROXIDASE-BLOCKING REAGENT, 10 mins	Ventana ultraview inhibitor
Wash sol	Phosphate buffer saline solution PBS (7.2-7.6)	Tris buffered saline with saponifying agent	EZ prep
Dilution of RTU	No	NA	No
Dilution of conc	NA	NA	NA
Diluent	NA	NA	NA
Inc time of Primary	1 hour 30 minutes covered in Humidity chamber	30 mins	1 hour
Detection	Polymer Based System	Polymer based system	Ventana ultraview kit (760-500)

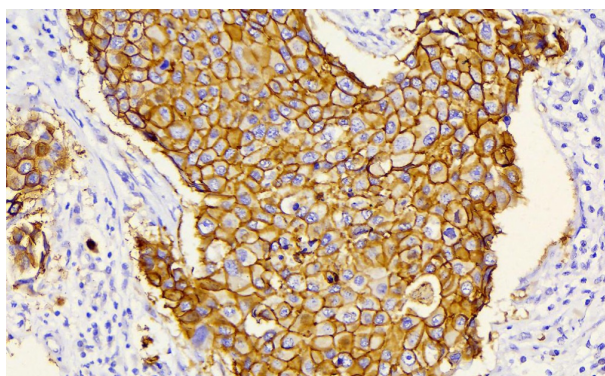
## General observations in Run 17



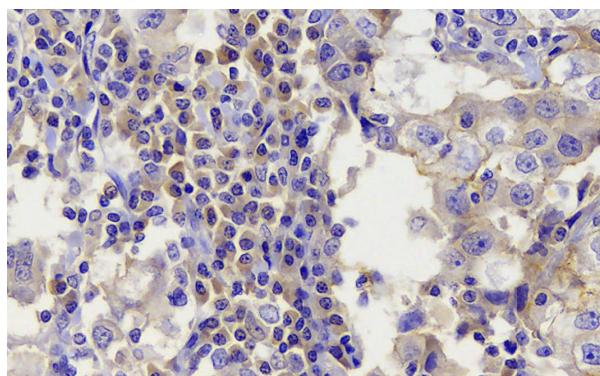
VARIABLE	1	2	3
Cat No	Super Sensitive Polymer HRP, IHC Detection System/Megavolume BiogeneX QD430-XAKEN, LOT: QD4300322, Expiry: June 2023	EnVision FLEX / HRP, Dako, K8023, 41388553, NA, 2023-01-31	Ventana ultraview kit (760-500), Ventana, cat no 760-500; H17947; 2022; 14/07/2023
Inc time of Sec	Polymer HRP-20 minutes Superenhancer-10 minutes At room temperature	30 mins	30min
Chro-substrate	DAB-10 minutes at room temperature	8 mins	7min
Post-treatment	No	NA	No
Counterstain	Harris Hematoxylin	Harris hematoxylin	Hematoxylin

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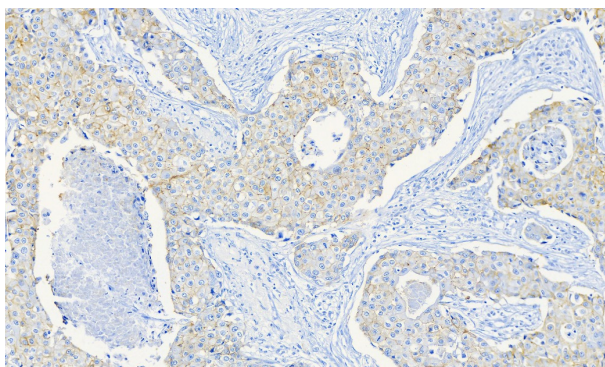




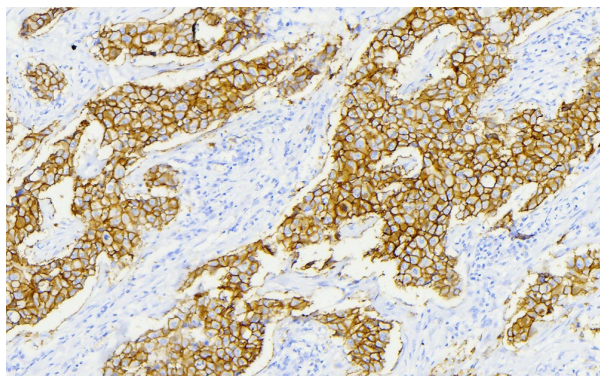
*Optimum staining of a positive case with FISH and 3+ IHC score*



*Non-specific cytoplasmic staining of tumor cells as well as the plasma cells in a participant*

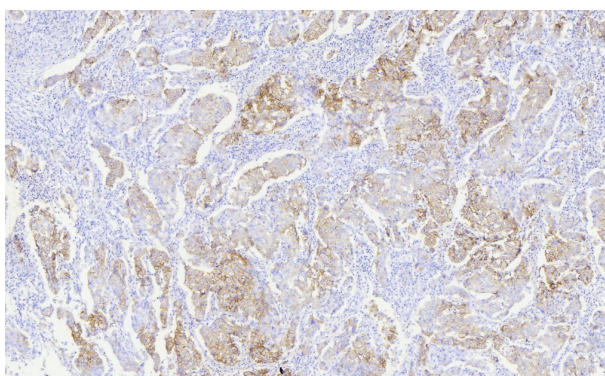


*Suboptimum staining of case 2*



*Optimum staining of case 2 with score 3*

:



*Staining with score 2+ in a case that is negative for most participant and also by FISH. Such cases would bear a comment "overstaining of a negative case", however it does not disqualify the protocol as this comment is with regards to comparison with FISH*