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Europe-side external quality assessment (EQA) of mRNA based testing of *ESR1*, *PGR*, *ERBB2*, and *MKI67* expression in invasive breast cancer

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Background:

Invasive breast cancer (IBC) subtypes, which are subject to different treatments, are identified in clinical routine by expression of estrogen receptor (ER), progesterone receptor (PR), Ki-67 and HER2 status by immunohistochemistry (IHC) and/or in situ hybridization (ISH). Yet, IHC evaluation might be hampered by (pre-)analytical errors and optimal cut-offs are still under discussion. Gene expression assays may offer a reliable way to measure mRNA expression of these four markers (*ESR1*, *PGR*, *ERBB2* and *MKI67*). Here, we investigated the correlation of the commercially available "four-marker" Xpert® Breast Cancer STRAT4 (CE-IVD)* mRNA assay with the gold standard (IHC/ISH) in different pathologic laboratories across Europe.

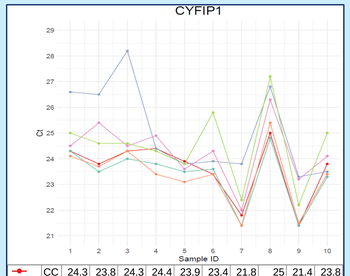
Design:

Ten pre-therapy breast core biopsies with IBC [six ER+/PR+ with varying Ki-67, two HER2+, two triple negative IBC diagnosed in the coordinating center (CC)] with sufficient formalin fixed paraffin embedded tissue were evaluated. IHC/ISH data for ER, PR, HER2 and Ki-67 were extracted from the original pathology report. For each case, STRAT4 (*ESR1*, *PGR*, *ERBB2* and *MKI67* mRNA assay) was performed in the CC and STRAT4 results matched IHC subtyping. Five European pathology laboratories participated in the harmonization study. Each site received one H&E stained slide and one unstained slide for STRAT4 testing. Binary mRNA results of each marker (positive vs. negative) were compared with the gold standard IHC/CISH of the CC. 80% of all results tested at each site had to be in agreement with the gold standard to pass the EQA.

	ER	PgR	HER2	Ki-67
sensitivity	1	0.94	1	0.86
specificity	1	1	1	1
precision (PPV)	1	1	1	1
accuracy	1	0.96	1	0.9

Table 1: Comparison of IHC/ISH status versus STRAT4 binary results – average performance across all five participating sites.

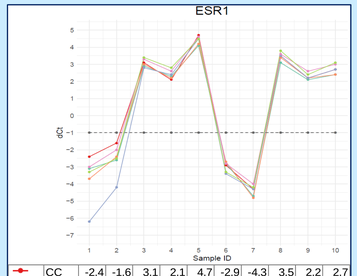
A



CC	24.3	23.8	24.3	24.4	23.9	23.4	21.8	25	21.4	23.8
A	24.3	23.5	24	23.8	23.5	23.6	21.4	24.8	21.4	23.3
B	24.1	23.7	24.3	23.4	23.1	23.4	21.4	25.4	21.5	23.4
C	26.6	26.5	28.2	24.4	23.8	23.9	23.8	26.8	23.3	23.5
D	24.5	25.4	24.5	24.9	23.6	24.3	22	26.3	23.2	24.1
E	25	24.6	24.6	24.3	23.8	25.8	22.4	27.2	22.2	25

All sites/all samples had CYFIP between 21-28 (well below valid Ct cutoff of 35)

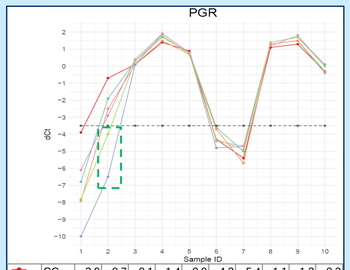
B



CC	-2.4	-1.6	3.1	2.1	4.7	-2.9	-4.3	3.5	2.2	2.7
A	-3.1	-2.6	2.9	2.3	4.1	-2.8	-4.7	3.1	2.1	2.4
B	-3.7	-2.4	3	2.2	4.2	-2.7	-4.8	3.4	2.2	2.4
C	-6.2	-4.2	2.8	2.4	4.5	-3.4	-4.3	3.5	2.2	2.7
D	-3	-2	3.3	2.6	4.6	-2.8	-4	3.6	2.6	3
E	-3.3	-2.5	3.4	2.8	4.5	-3.3	-4.2	3.8	2.4	3.1
cut-off	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
IHC (%)	0	0	95	90	95	0	0	80	95	95

100% accuracy with comparable *ESR1* dCt measurements across sites for all samples

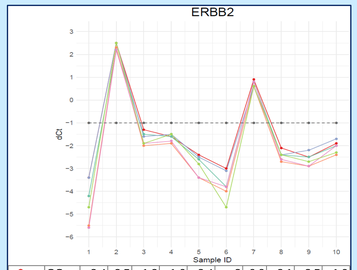
C



CC	-3.9	-0.7	0.1	1.4	0.9	-4.3	-5.4	1.1	1.3	-0.3
A	-6.8	-1.9	0.3	1.7	0.8	-3.6	-5	1.3	1.5	-0.3
B	-7.9	-2.5	0.1	1.5	0.7	-3.7	-5.7	1.3	1.5	-0.4
C	-10	-6.5	0.1	1.9	0.8	-4.8	-4.7	1.4	1.7	0.1
D	-6.1	-2.9	0.4	1.9	0.8	-4.4	-4.7	1.2	1.8	0
E	-7.8	-4	0.3	1.8	0.7	-4.3	-4.9	1.4	1.7	0
cut-off	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5
IHC (%)	0	<10	10	90	<10	0	0	70	60	100

One false negative sample in 2 sites

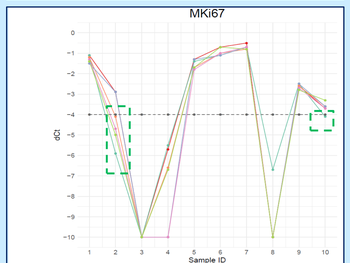
D



CC	-3.4	2.5	-1.3	-1.6	-2.4	-3	0.9	-2.1	-2.5	-1.9
A	-4.2	2.2	-1.5	-1.6	-2.6	-3.8	0.8	-2.4	-2.5	-2
B	-5.5	2.3	-2	-1.9	-3.4	-4	0.6	-2.7	-2.9	-2.4
C	-3.4	2.5	-1.6	-1.5	-2.5	-3.1	0.6	-2.4	-2.2	-1.7
D	-5.6	2.2	-1.9	-1.8	-3.4	-3.8	0.8	-2.6	-2.9	-2
E	-4.7	2.5	-1.9	-1.5	-2.8	-4.7	0.6	-2.4	-2.7	-2.3
cut-off	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
IHC (Dako Score)	1	3	1	1	0	1	3	0	2	1

100% accuracy with comparable dCt measurements across sites for all samples (#9: CISH neg.)

E



CC	-1.1	-2.9	-10	-5.7	-1.3	-0.7	-0.5	-10	-2.6	-3.7
A	-1.1	-5.9	-10	-5.5	-1.4	-1.1	-0.7	-6.7	-2.5	-4.1
B	-1.2	-4.1	-10	-6.6	-1.7	-1	-0.8	-10	-2.6	-3.6
C	-1.5	-2.9	-10	-10	-1.3	-1.1	-0.7	-10	-2.5	-3.6
D	-1.3	-4.7	-10	-10	-1.8	-1	-0.7	-10	-2.7	-3.7
E	-1.4	-5	-10	-6.7	-1.7	-0.7	-0.8	-10	-2.8	-3.3
cut-off	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4
IHC (%highest)	30	30	5	15	50	80	50	15	30	30

One false negative sample in 4 sites and another false negative in 1 site

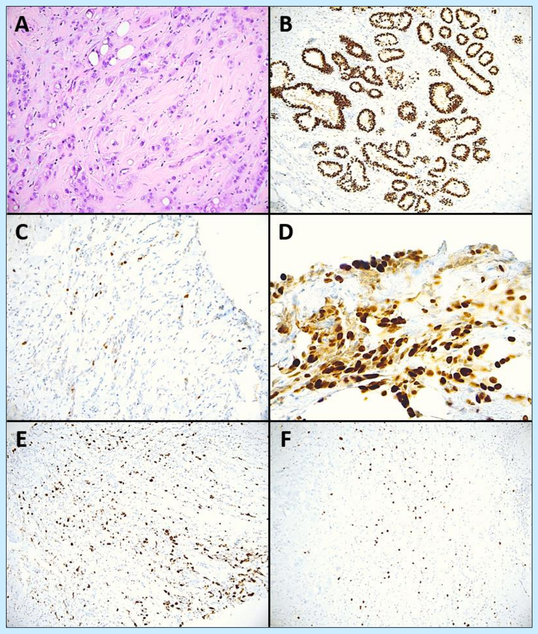
Figure 1:
A) Cytoplasmic FMR1-Interacting Protein 1 (CYFIP1) cycle threshold (Ct) trends across the coordination center and the five participating sites A-E.
B) - E)
Delta cycle threshold (dCt) trends across the coordination center and the five participating sites A-E
B) Estrogen receptor (*ESR1*)
C) Progesterone receptor (*PGR*)
D) Human epidermal growth factor receptor (*ERBB2*)
E) Ki-67 (*MKI67*)

Figure 2: Case #2 is a pleomorphic invasive lobular breast cancer, which is illustrated in hematoxylin and eosin staining in A) (x200).

B) shows the internal positive control (non-neoplastic breast epithelium) in progesterone receptor (PgR) immunohistochemistry (IHC) (x100).

In C) (x200) and D) (x400), heterogeneous IHC expression of PgR (mostly negative, but focally positive with strong intensity) can be seen.

E) and F) illustrate the heterogeneous IHC expression of Ki-67 (x100).



Results:

All centers passed the EQA study. Sensitivity, specificity and accuracy of *ESR1* and *ERBB2* mRNA STRAT4 testing were 100% for all ten samples. Instead, *PGR* was falsely reported as negative for one case (case #2) by two sites and *MKI67* was falsely negative for two cases (case #2 by four sites, #10 by one site) (Table 1, Figure 1). Case #2 was a pleomorphic invasive lobular BC with heterogeneous low positive PgR (IHC staining <10%) and heterogeneous Ki-67 IHC (up to 30%) (Figure 2). A second STRAT4 analysis of deeper tumor block sections in the CC confirmed *PGR* (dCt = -1.5) and *MKI67* positivity (dCt = -3.2). Case #10 showed a partly inhomogeneous Ki-67 IHC expression. Unexpectedly, repetition of STRAT4 testing in the CC delivered a negative *MKI67* status (dCt = -4.1), which was, however, very close to the cut-off (dCt = -4.0).

Conclusion:

The results of our study showed that STRAT4 might offer a reliable alternative for the evaluation of ER, PR, HER2 and Ki-67 in IBC. However, prognostic and predictive value of STRAT4 should be further validated in clinical cohorts.

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*CE-IVD. In vitro diagnostic medical device. Not available in all countries. Not available in the US.