GeneXpert
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Xpert® Breast Cancer STRAT4

REF GXBCSTRAT4-CE-10

Instructions for Use

CE IVD

Cepheid

In Vitro Diagnostic Medical Device

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Trademark, Patents and Copyright Statements

See Section 24 Revision History, for a description of changes.

Xpert® Breast Cancer STRAT4

In Vitro Diagnostic Medical Device

1 Proprietary Name

Xpert® Breast Cancer STRAT4

2 Common or Usual Name

Xpert Breast CA STRAT4

Xpert BC STRAT4

3 Intended Use

Xpert Breast Cancer STRAT4 test is a polymerase chain reaction-based semi-quantitative assay with qualitative cut-off values for Estrogen Receptor (*ESR1*), Progesterone Receptor (*PGR*), Human Epidermal Growth Factor Receptor 2 (*ERBB2/HER2*), and Marker of Proliferation Ki-67 (*MKi67*) mRNAs isolated from formalin-fixed paraffin-embedded (FFPE) invasive breast cancer tissue. The RNA is extracted from a tumor-enriched area of a microscope tissue section as identified by a pathologist. The test is to be used in combination with other clinical and laboratory data to classify breast cancer tissues regarding their hormone receptor status, the HER2 receptor status, and the Proliferation marker status. The test is intended to be used with the GeneXpert® system, which includes RNA isolation from FFPE tissue, as well as amplification and detection of target sequences within the cartridge.

The Xpert Breast Cancer STRAT4 test is not intended as:

- A predictor of disease severity
- A stand-alone device for diagnostic testing for breast cancer
- A prognosticator for disease recurrence

Indications for Use: The test is intended for use in assessing the mRNA levels of *ESR1*, *PGR*, *ERBB2*, and *MKi67* in invasive breast cancer tissues obtained from patients and prepared as FFPE specimens, and as an aid in clinical evaluation in conjunction with other laboratory data.

4 Summary and Explanation

Breast cancer is one of the most common cancers in women worldwide, with approximately 1.7 million new breast cancer cases every year.[1] In Europe, approximately 494,000 new cases are diagnosed each year and 143,000 patients will die of their disease. In the US, approximately 200,000 new cases of invasive breast cancer were diagnosed in 2015.[2] Breast cancer is the most common cause of cancer mortality among women in developing countries and the second most common cause of cancer mortality (after lung cancer) among women in developed countries.[2]

In women, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death.[1] Breast cancer mortality has decreased by 34 percent since 1990 largely due to improved treatment and early detection.[3] Measurements of ER and PR protein expression are prognostic for breast cancer outcomes and they predict response to tamoxifen and other hormonal therapies.[4,5,6,7] HER2 overexpression conveys an adverse prognosis in women with breast cancer; but more importantly, response to trastuzumab or other HER2-targeted therapies is predicted by HER2 (ERBB2) protein overexpression or HER2 gene amplification.[8] Marker of Proliferation Ki-67 (MKi67) has been widely studied in retrospective studies involving breast cancer patients[9] and is considered an important indicator of the need for chemotherapy.[10] Meta-analyses have demonstrated that it is associated with worse survival outcomes in early breast cancer.[11]

Given these markers' importance in the selection of an effective treatment regimen for a patient with breast cancer, the European Society for Medical Oncology (ESMO) treatment guidelines recommend that all primary breast carcinomas be tested for ER, PR, HER2 (ERBB2), and Ki67 at the time of diagnosis.[12]

Immunohistochemistry (IHC) is commonly used for the measurement of ER, PR, HER2 and Ki67 protein expression. For HER2 expression, IHC is typically the first test performed and results are reported on a scale of 0 to 3+. If the result is equivocal for HER2 expression (2+), the sample is reflexed to a HER2 in situ hybridization (ISH) assay, such as fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) that looks for HER2 gene amplification.[13] A high degree of variability in results has been shown for IHC and ISH when compared across laboratories, largely due to differences in the antibodies used for IHC as well as subjectivity of interpretation methods.[14]

The Xpert Breast Cancer STRAT4 test is an in vitro diagnostic test used to determine the mRNA expression levels of *ESR1*, *PGR*, *ERBB2*, and *MKi67* isolated from FFPE specimens of invasive breast cancer tissue.

The assay is performed in a self-contained cartridge following a brief off-board sample lysate preparation step, requiring less than 15 minutes of hands-on time with a total turn-around-time of less than 2 hours.

5 Principle of the Procedure

The Xpert Breast Cancer STRAT4 test is a real-time Polymerase Chain Reaction (PCR) assay for the detection of ESR1, PGR, ERBB2, and MKi67 mRNAs isolated from formalin-fixed paraffin-embedded (FFPE) invasive breast tissue. The assay is performed on the Cepheid GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and target sequence detection in simple or complex samples using real-time RT-PCR. The systems consist of an instrument, barcode scanner, computer, and pre-loaded software for running tests and viewing the results. The systems employ single-use, disposable GeneXpert cartridges that hold the RT-PCR reagents and host the RT-PCR process. For a full description of the systems, refer to the appropriate GeneXpert Instrument System Operator Manual.

The Xpert Breast Cancer STRAT4 test includes reagents for the simultaneous detection of ESR1, PGR, ERBB2, MKi67, a cytoplasmic FMR1 interacting protein 1 (CYFIP1) reference gene, an internal RT-PCR control (CIC), and an internal Probe Check Control (PCC). The reference gene verifies specimen adequacy and is used to normalize the mRNA expression levels for ESR1, PGR, ERBB2, and MKi67. The internal RT-PCR control (CIC) is used to verify that the RT-PCR reaction proceeded correctly. The PCC verifies reagent bead rehydration, RT-PCR tube-filling, probe integrity and dye stability in the cartridge. In total, the assay utilizes six distinct fluorescent channels for target or control/reference detection with its own cut-off parameters for target/control/reference validity.

FFPE samples must first be treated with the Xpert® FFPE Lysis Kit by preparing a 4-5 µm (micron)-thick tissue section where the FFPE tissue is first macro-dissected, if required, to enrich invasive tumor area, and then scraped and placed into a tube along with the recommended volumes of FFPE lysis reagent and proteinase K. The solution is then incubated in a heat block at 80 °C for 30 minutes. Ethanol is then mixed with the sample and the recommended volume of the prepared sample lysate is then added directly to a test cartridge. The testing cartridge is inserted into a module of a GeneXpert Instrument System where nucleic acid purification, amplification, and real-time detection are all fully automated and completely integrated by the system. All reagents required for onboard sample preparation and RT-PCR analysis are preloaded in the cartridge. Nucleic acids in the lysate are captured on a filter, washed, and eluted by sonication. The purified nucleic acid is mixed with dry RT-PCR reagents, and the solution is transferred to the reaction tube for RT-PCR and detection. Time to result is approximately 75 minutes in the GeneXpert.

The detection cut-offs that the Xpert Breast Cancer STRAT4 test utilizes in each fluorescent channel were established to maximize positive, negative, and overall percent agreement compared to reference lab IHC or IHC/FISH results for each target. IHC for ER, PR, Ki67, and HER2 as well as FISH for HER2 were processed and scored per Instructions for Use instructions. Interpretation of results was completed per ASCO/CAP 2013 guidelines.[15] Tumors were classified as ER or PRIHC positive when ≥1% of invasive tumor cells showed definite nuclear staining, irrespective of staining intensity. HER2 expression was evaluated with the HercepTest (IHC) kit (Dako) and scored as 0, 1+, 2+, or 3+. Tumors scored as 2+ were reflexed to HER2 FISH using the PathVysion HER2 DNA probe kit (Vysis-Abbott, Chicago, IL). Cases were considered HER2-positive if scored 3+ by IHC and/or amplified by FISH (defined as HER2:CEP17 (ratio ≥ 2.0), and/or average HER2 copy number ≥ 6.0 signals/cell according to the 2013 ASCO/CAP Clinical Practice Guideline Update for HER2 Testing in Breast Cancer.[15] For Ki67, tumors were classified as positive (high) when ≥20% of invasive tumor cells showed definite nuclear staining, irrespective of staining intensity.

In the case of the reference gene control and internal RT-PCR control, the detection cut-offs define ranges of minimum and maximum cycle threshold (Ct) PCR values that determine a valid result, an adequate minimum sample input, and no PCR inhibition. In the case of the ESR1, PGR, ERBB2, and MKi67 targets, the detection cut-offs are defined by delta cycle threshold (dCt) (reference gene Ct minus target gene Ct) values that determine POSITIVE vs. NEGATIVE results for a given target in a channel.

6 Reagents and Instruments

6.1 Material Provided

The Xpert Breast Cancer STRAT4 kit contains sufficient reagents to process 10 quality control samples or FFPE lysates prepared with the Xpert FFPE Lysis Kit (catalog# GXFFPE-LYSIS-CE-10). The Xpert Breast Cancer STRAT4 kit contains the following items:

Xpert Breast Cancer STRAT4 Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, 2, and 3 (freeze-dried)	1 per cartridge
• Rinse Reagent,	1.0 mL per cartridge
• Elution Reagent,	2.0 mL per cartridge
CD	**1 per kit**

- Assay Definition File (ADF)
- Instructions for Use

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no commingling of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Breast Cancer STRAT4 kit contents at 2–28 °C.
- Do not open the cartridge lid until you are ready to perform testing.
- Use the cartridge within 30 minutes after opening the lid.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- Xpert FFPE Lysis Kit (catalog# GXFFPE-LYSIS-CE-10) for preparing FFPE lysate. This kit consists of FFPE Lysis Reagent, Proteinase K (PK), 1.5 mL tubes, and 5 mL vials.
- Vortex mixer.
- Pipettes and aerosol filter pipette tips suitable to pipette 600uL 1.2 µL and 520 µL.
- Computer with proprietary GeneXpert software version 4.7b or higher or Xpertise Version 6.4b or higher, barcode scanner and appropriate GeneXpert Instrument System operator manual.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

9 Warnings and Precautions

- For *In Vitro* Diagnostic Use Only.
- All biological samples should be treated as if they are capable of transmitting infectious agents. All human samples should be treated with standard precautions. Guidelines for specimen handling are available from the World Health Organization or U.S. Centers for Disease Control and Prevention.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Performance characteristics of this test have been established with the specimen type listed in Section 3. The performance of this assay with other specimen types or samples has not been evaluated.
- FFPE tissue must be processed with Xpert FFPE Lysis Kit (catalog# GXFFPE-LYSIS-CE-10).

- Incomplete removal (scraping) of tumor area from the slide for preparation of the FFPE lysate may result in insufficient material for the assay and therefore a higher than expected indeterminate/Invalid rate with the Xpert Breast Cancer STRAT4 test.
- Do not open the Xpert Breast Cancer STRAT4 cartridge lid except when adding prepared FFPE lysate.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Breast Cancer STRAT4 cartridge is used to process one test. Do not reuse spent cartridges.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not place the sample ID label on the cartridge lid or on the bar code label.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents. Check state, territorial, or local regulations as they may differ from national disposal regulations. The material may exhibit characteristics of hazardous waste requiring specific disposal requirements. Institutions should check their hazardous waste disposal requirements.

10 Chemical Hazards[16,17]

According to the Globally Harmonized System for Classification and Labeling (GHS), this material is not considered hazardous.

11 Specimen Collection, Transport, and Storage

- Use only with FFPE specimens processed with the Xpert FFPE Lysis Kit (catalog# GXFFPE-LYSIS-CE-10). Follow ASCO/CAP guidelines[15] for preparing FFPE tissue.
- FFPE lysate should be prepared from the FFPE tumor block with the greatest area of viable breast carcinoma (a minimum of 30% tumor cellularity) and manual macro-dissection should be performed, if required, prior to testing in the Xpert Breast Cancer STRAT4 test. For tumor samples less than 10 mm^2 with less than 30% tumor, use of the concentrated lysate procedure or more than one 4-5 µm section may be required for valid results.
- FFPE lysate should be transported to the laboratory at 2–8 °C.
- FFPE lysate is stable up to 1 week at 2–8 °C or 4 weeks at ≤ -20 ºC before testing with Xpert Breast Cancer STRAT4. For long term storage, store at -80 ºC. No more than 1 freeze-thaw is recommended. When thawing, please thaw to room temperature and vortex FFPE lysate for 15 seconds prior to use.

12 Procedure

Important **Use of the Xpert Breast Cancer STRAT4 cartridge requires preparation of a lysate using Xpert FFPE Lysis Kit (catalog# GXFFPE-LYSIS-CE-10).**

Important **Start the assay within 30 minutes of adding the prepared sample to the cartridge.**

12.1 Preparing the FFPE Lysate

Prepare FFPE lysate per FFPE Lysis Kit Instructions for Use.

12.2 Preparing the Cartridge

1. Remove the cartridge from the cardboard packaging.
2. Vortex prepared FFPE lysate 15 seconds prior to use.
3. Open the cartridge lid.
4. Using a pipette, transfer 520 µL of FFPE lysate to the Sample Chamber of the cartridge. (Note: a small amount of precipitate may be present, which does not affect assay performance).

   Retain remaining FFPE lysate at 2–8 °C or ≤–20 °C in case of retest.

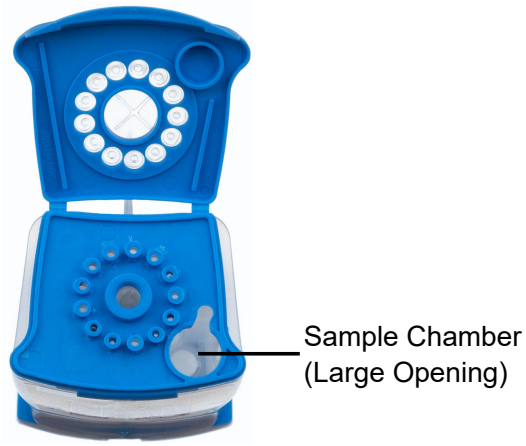


Figure 1. Xpert Breast Cancer STRAT4 Cartridge (Top View)

5. Close the cartridge lid. Ensure the lid snaps firmly into place.

12.3 Starting the Test

Important **Before starting the test, make sure the Xpert Breast Cancer STRAT4 Assay Definition File (ADF) is imported into the software.**

This section lists the default steps to operate the GeneXpert System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the instrument that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert instrument:
   - If using the GeneXpert Dx instrument, first turn on the GeneXpert Dx instrument, and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software icon on the Windows® desktop.

     or
   - If using the GeneXpert Infinity instrument, power up the instrument. The Xpertise software will launch automatically or may require double-clicking the Xpertise software icon on the Windows desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (Infinity). The Create Test window opens.
3. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports. The Scan Cartridge dialog box appears.
4. Scan the barcode on the Xpert Breast Cancer STRAT4 cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN.
5. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Enter your password, if requested.
6. For the GeneXpert Dx Instrument:
   a) Open the instrument module door with the blinking green light and load the cartridge.
   b) Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
   c) Wait until the system releases the door lock before opening the module door. Remove cartridge.
   d) Dispose of used cartridges in the appropriate specimen waste containers according to your institution's standard practices. See Section 9.

   or

   For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

13 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions concerning how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending upon the instrument used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button on the View Results screen to view and/or generate a PDF report file.

14 Quality Control

Each test contains a Reference Gene Control (*CYFIP1*) and a Probe Check Control (PCC).

- ***CYFIP1* Control**: This reference gene is used to normalize the expression levels for *ESR1*, *PGR*, *ERBB2*, and *MKi67*. It also serves as a Sample Adequacy Control (SAC) ensuring that the sample contains sufficient RNA. A minimum *CYFIP1* signal is required for a valid test result. A *CYFIP1* signal below the minimum amount or a negative signal indicates that the sample does not contain sufficient RNA.
- ***CYFIP1* Alternate**: This is a duplicate *CYFIP1* control used in the algorithm when delta cycle threshold (dCt) of PGR or *MKi67* is below the assay cutoff setting. For these targets, an additional minimum *CYFIP1* alternate signal is needed to ensure a valid test result.
- **Probe Check Control (PCC):** Before the start of the PCR, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability. The PCC passes if it meets the validated acceptance criteria.
- **External Controls (not supplied):** External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.

15 Interpretation of Results

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window on the Test Results and Analyte Result tabs. The Test Result and Analyte Results are also shown on the Test Report. The possible results are shown in Table 1 and Table 2.

Table 1. All Possible Results for the Xpert Breast Cancer STRAT4 Test

Result Displayed	CYFIP1	CYFIP1 Alternate	CIC
ESR1 POSITIVE	PASS	POS or NEG	POS or NEG
ESR1 NEGATIVE	PASS	POS or NEG	POS or NEG
PGR POSITIVE	PASS	POS or NEG	POS or NEG
PGR NEGATIVE	PASS	POS	POS or NEG
ERBB2 POSITIVE	PASS	POS or NEG	POS or NEG
ERBB2 NEGATIVE	PASS	POS or NEG	POS or NEG
MKi67 POSITIVE	PASS	POS or NEG	POS or NEG
MKi67 NEGATIVE	PASS	POS	POS or NEG
PGR INDETERMINATE	PASS	NEG	POS or NEG
MKi67 INDETERMINATE	PASS	NEG	POS or NEG
REPEAT TEST	PASS	POS or NEG	NEG
INVALID	FAIL	NEG	POS or NEG
ERROR	NO RESULT	NO RESULT	NO RESULT
NO RESULT	NO RESULT	NO RESULT	NO RESULT

Table 2. Xpert Breast Cancer STRAT4 Representative Results and Interpretation

Result	Interpretation
ESR1 POSITIVE See Figure 2.	• *ESR1* mRNA transcript is overexpressed and has a delta cycle threshold (dCt) above the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
PGR POSITIVE See Figure 2.	• *PGR* mRNA transcript is overexpressed and has a delta cycle threshold (dCt) above the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
ERBB2 POSITIVE See Figure 2.	• *ERBB2* mRNA transcript is overexpressed and has a delta cycle threshold (dCt) above the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
MKi67 POSITIVE See Figure 2.	• *MKi67* mRNA transcript is overexpressed and has a delta cycle threshold (dCt) above the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
ESR1 NEGATIVE See Figure 3.	• *ESR1* mRNA transcript is not overexpressed and has a delta cycle threshold (dCt) below the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.

Result	Interpretation
PGR NEGATIVE See Figure 3.	• *PGR* mRNA transcript is not overexpressed and has a delta cycle threshold (dCt) below the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • *CYFIP1* alternate – POS; *CYFIP1* has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
ERBB2 NEGATIVE See Figure 3.	• *ERBB2* mRNA transcript is not overexpressed and has a delta cycle threshold (dCt) below the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
MKi67 NEGATIVE See Figure 3.	• *MKi67* mRNA transcript is not overexpressed and has a delta cycle threshold (dCt) below the cutoff setting. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • *CYFIP1* alternate – POS; *CYFIP1* has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • Probe Check – PASS; all probe check results passed.
PGR Indeterminate See Figure 4.	• *PGR* mRNA expression level cannot be determined due to sample containing insufficient material. Repeat the test using a more concentrated lysate. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • *CYFIP1* alternate – NEG; *CYFIP1* cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting necessary for PGR status determination. • Probe Check – PASS; all probe check results passed.
MKi67 Indeterminate See Figure 4.	• *MKi67* mRNA expression level cannot be determined due to sample containing insufficient material. Repeat the test using a more concentrated lysate. • CYFIP1 – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • CYFIP1 alternate – NEG; *CYFIP1* cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting necessary for MKi67 status determination. • Probe Check – PASS; all probe check results passed.
REPEAT TEST See Figure 5.	• *ESR1/PGR/ERBB2/MKi67* mRNA expression levels cannot be determined. Repeat the test using an aliquot of retained FFPE sample lysate. • *CYFIP1* – PASS; *CYFIP1* mRNA transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • *CYFIP1* alternate – POS/NEG; *CYFIP1* mRNA transcript was detected. The transcript may or may not have a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. • CIC – NEG; internal control has a cycle threshold (Ct) outside the valid range. • Probe Check – PASS; all probe check results passed.

Result	Interpretation
INVALID	• INVALID – *ESR1/PGR/ERBB2/MKi67* mRNA expression levels cannot be determined due to sample containing insufficient material. Repeat the test using a more concentrated lysate. • *CYFIP1* – FAIL; *CYFIP1* cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting. • *CYFIP1* alternate – NEG; *CYFIP1* cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting. • Probe Check – PASS; all probe check results passed.
ERROR	• *ESR1/PGR/ERBB2/MKi67* mRNA expression levels cannot be determined. Repeat the test using an aliquot of retained FFPE sample lysate. • *ESR1/PGR/ERBB2/MKi67* – NO RESULT • *CYFIP1/CYFIP1* alternate – NO RESULT • Probe Check – PASS*/FAIL; all or one of the probe check results failed. * If the probe check passed, the error was caused by the maximum pressure limit exceeding the acceptable range, a curve fit error or by a system component failure.
NO RESULT	• *ESR1/PGR/ERBB2/MKi67* mRNA expression levels cannot be determined. Insufficient data were collected to produce a test result. For example, this can occur if the operator stopped a test that was in progress. Repeat the test using retained FFPE sample lysate. • *ESR1/PGR/ERBB2/MKi67* – NO RESULT • *CYFIP1/CYFIP1* alternate – NO RESULT • Probe Check – NA (not applicable)

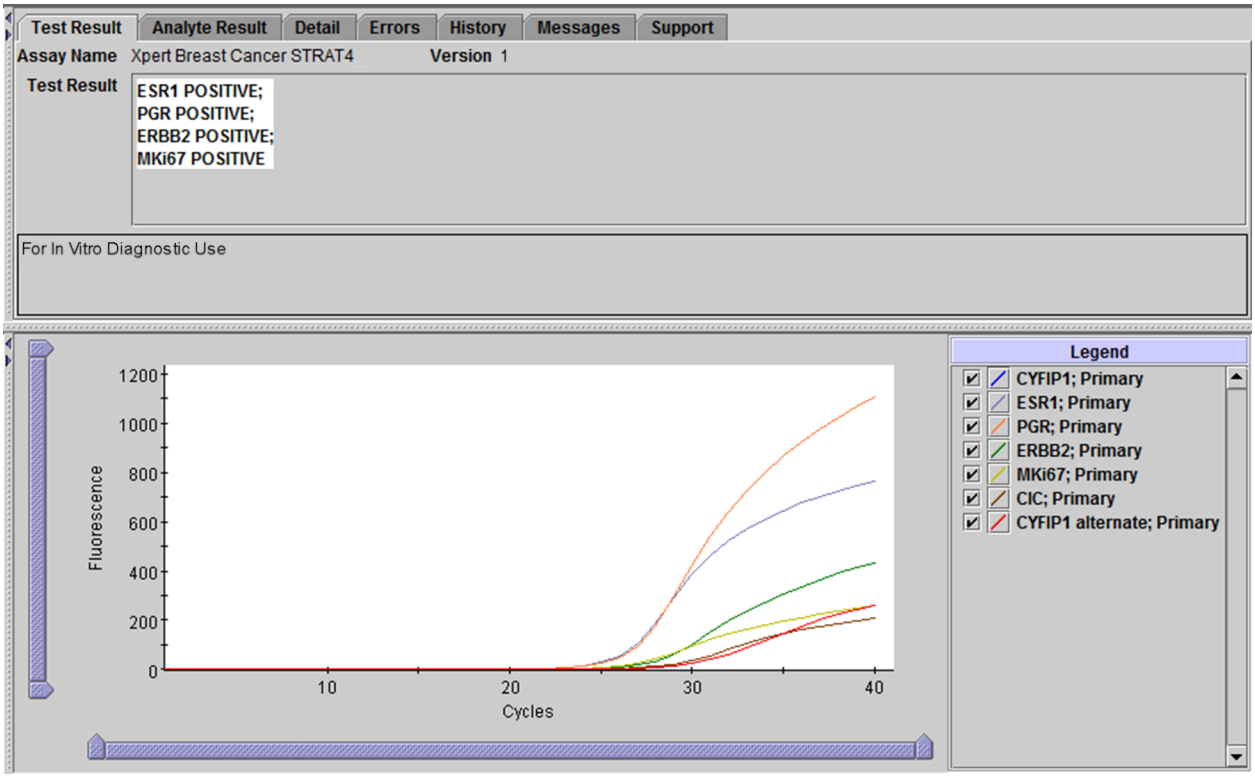


Figure 2. GeneXpert Dx View Results Window: ESR1/PGR/ERBB2/MKi67 POSITIVE

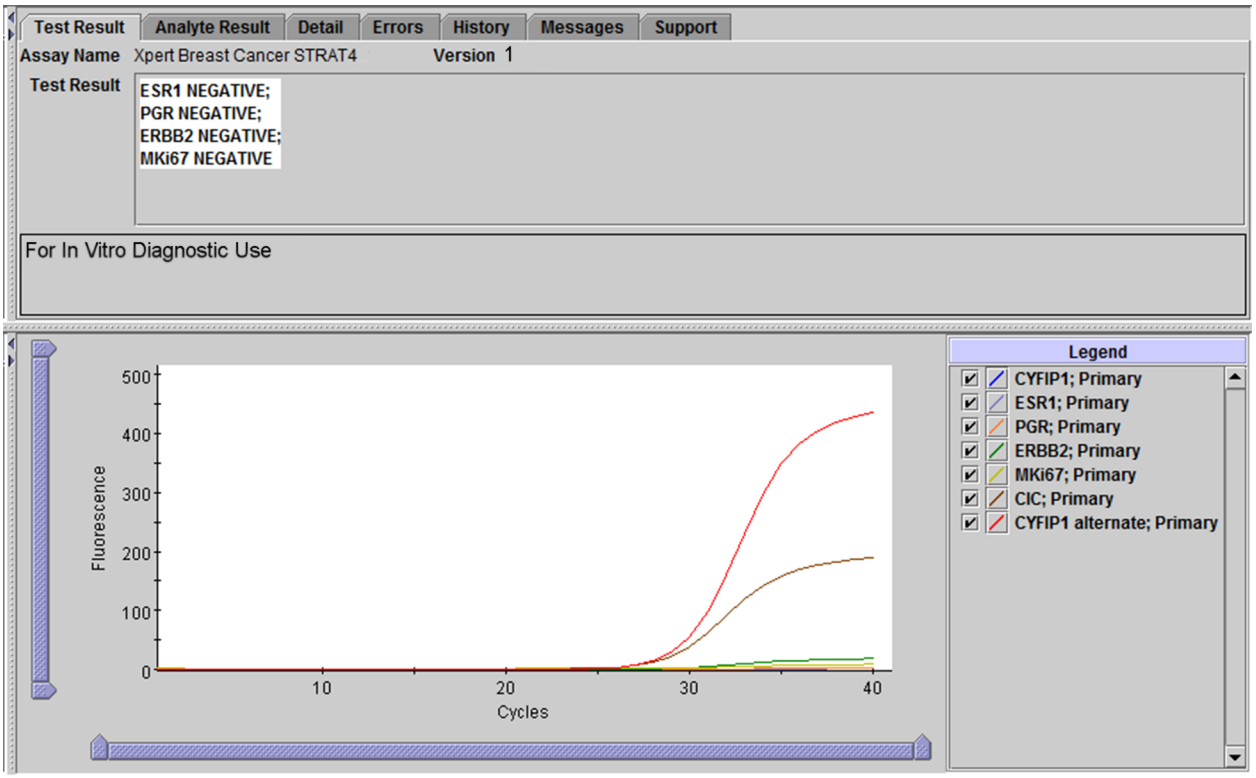


Figure 3. GeneXpert Dx View Results Window: ESR1/PGR/ERBB2/MKi67 NEGATIVE

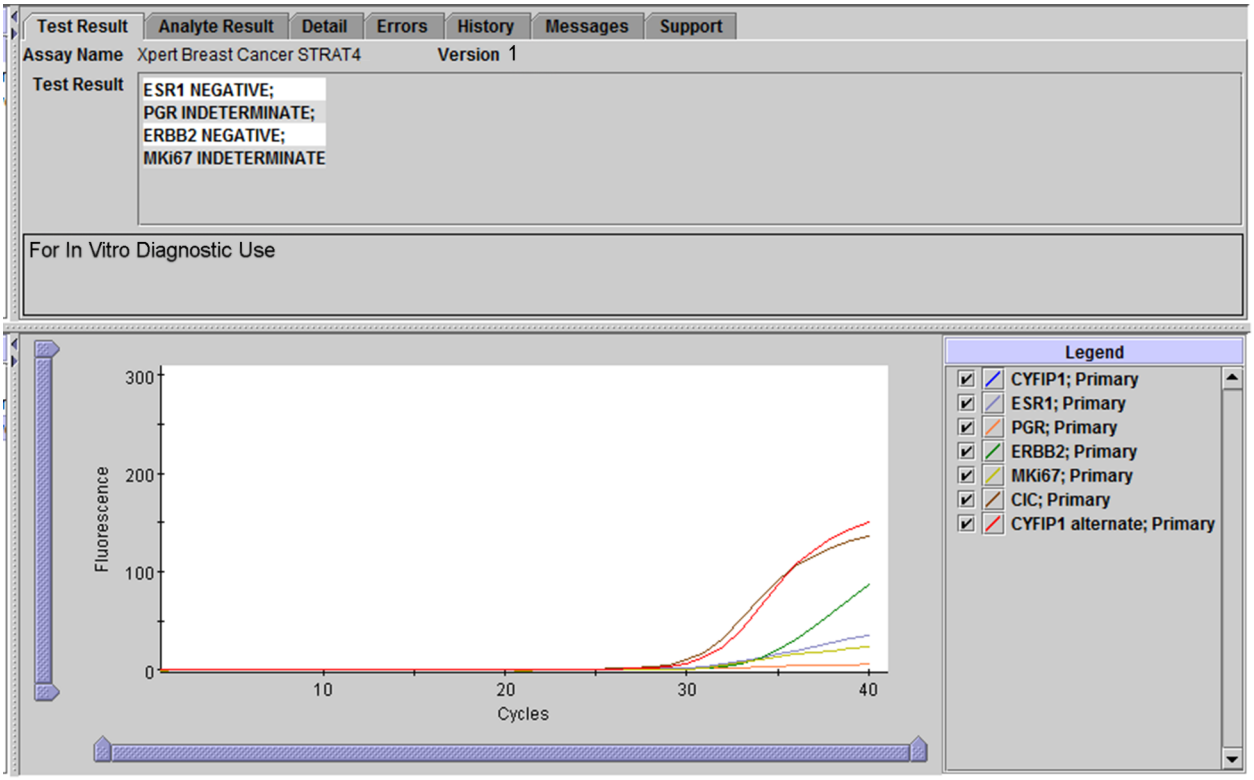


Figure 4. GeneXpert Dx View Results Window: PGR/MKi67 INDETERMINATE

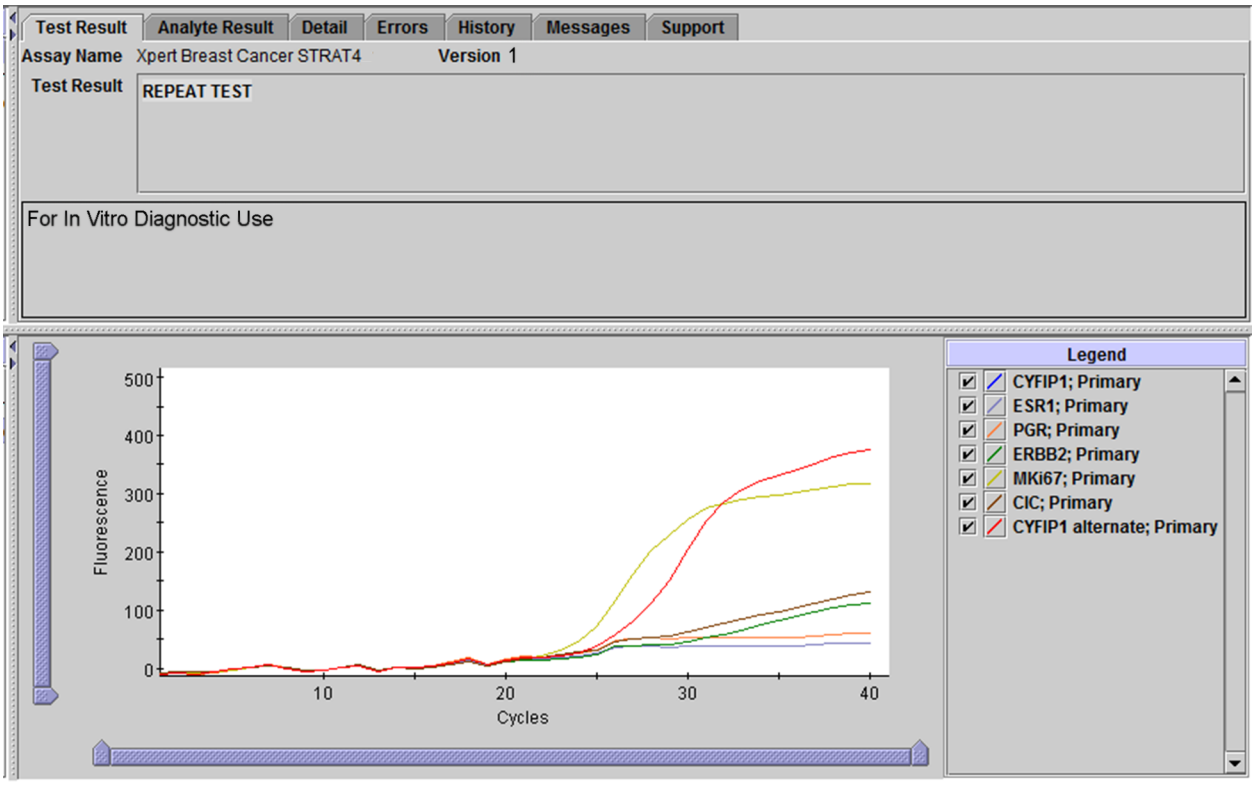


Figure 5. GeneXpert Dx View Results Window: REPEAT TEST

16 Reasons to Repeat the Test

Repeat the test using a new cartridge (do not re-use the cartridge).

- A **REPEAT TEST** result indicates that the internal control failed. The sample was not properly processed. In this case, repeat the test using a new 520 µL aliquot of the same FFPE lysate.
- An **INVALID** result indicates that the reference control failed. The sample was not properly processed, the PCR was inhibited, or the RNA quality in the tumor accessed was inadequate. In this case, repeat the test with a more concentrated FFPE lysate per FFPE Lysis Kit Instructions for Use instructions.
- An **ERROR** result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, because the maximum pressure limits were exceeded, or a valve positioning error was detected. In this case, repeat the test using a new 520 µL aliquot of the same FFPE lysate.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred. In this case, repeat the test using a new 520 µL aliquot of the same FFPE lysate.
- If an External QC fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

17 Limitations

- Modifications to these procedures may alter the performance of the test. Results from Xpert Breast Cancer STRAT4 should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- The performance of Xpert Breast Cancer STRAT4 was validated using the procedures provided in this instruction for use and using FFPE specimens that were five to ten years old.
- The performance of the Xpert Breast Cancer STRAT4 was validated using the procedures provided in this instruction for use only.

- Erroneous test results might occur from improper specimen collection, handling, storage, or sample mix-up. Careful compliance to the instructions in this instruction for use is necessary to avoid erroneous results.
- Performance characteristics were not established for patients less than 25 years of age.
- Mutations or polymorphisms in primer or probe binding regions may result in erroneous but believable results for *ESR1*, *PGR*, *ERBB2*, and *MKi67*.

18 Performance Characteristics

18.1 Clinical Performance

Performance characteristics for the Xpert Breast Cancer STRAT4 test were evaluated relative to IHC results for ER, PR, HER2, and Ki67 and to fluorescence *in situ* hybridization (FISH) for HER2 gene amplification at sites in the US and EU. Initially, a total of 211 de-identified leftover FFPE specimens of primary invasive breast cancer tumors from the US and EU were enrolled in this study. 10 specimens were excluded because insufficient tumor was available for testing, and one specimen was excluded due to retracted consent. Thus, a total of 200 specimens were available for inclusion in the data analyses. For each FFPE specimen, multiple slides were prepared for testing by Xpert; for IHC testing of ER, PR, HER2, and Ki67; and for FISH testing of HER2 gene amplification.

Overall, Xpert Breast Cancer STRAT4 provided valid results on the first testing attempt for 99.5% (199/200) of study specimens. One specimen initially yielding a non-determinate result (**ERROR**, **INVALID** or **NO RESULT**) gave a test result after a single retest. The overall assay success rate was 100.0% (200/200).

Of the 200 specimens with valid Xpert test results, ESR1 and ERBB2 gave a valid positive or negative test result 100% of the time (200/200). For PGR and MKi67, Xpert gave a valid positive or negative test result in 98.5% (197/200) and 97.0% (194/200) of the cases, respectively. The 7 specimens with Xpert indeterminate results for PGR and/or MKi67 were retested using the concentrated FFPE lysate method. Both the original (first attempt) and retest results are shown in Table 3.

For the entire dataset, including the re-test results, Xpert Breast Cancer STRAT4 demonstrated a Positive Percent Agreement (PPA) of 97.2%, a Negative Percent Agreement (NPA) of 95.0%, and an Overall Percent Agreement (OPA) of 97.0% for ESR1 relative to IHC;[18]PPA of 88.4%, NPA of 90.7%, and OPA of 88.9% for PGR relative to IHC;[18] PPA of 100.0%, NPA of 92.4%, and OPA of 93.3% for ERBB2 relative to IHC;[19] and PPA of 100%, NPA of 92.0%, and OPA of 93.3% for ERBB2 relative to HER2 FISH.[19] For MKi67 a PPA of 88.8%, NPA of 100%, and OPA of 90.7% with the IHC threshold set at>20% for positive and <10% for negative. MKi67 IHC intermediate specimens (10%-20% threshold, inclusive) were excluded from the analysis. The overall PPA, NPA and OPA for each target are shown in Table 3.

Table 3. Clinical Performance

Comparison	Data Set[a]	Total (n)[b]	PPA	95% CI	NPA	95% CI	OPA	95% CI
ESR1/ER Xpert vs. IHC	Original	199	97.2% (174/179)	93.6-98.8	100% (20/20)	83.9-100	97.5% (194/199)	94.3-98.9
	Retest	199	97.2% (174/179)	93.6-98.8	95.0% (19/20)	76.4-99.1	97.0% (193/199)	93.6-98.6
PGR/PR Xpert vs. IHC	Original	196	89.0% (137/154)	83.0-93.0	92.9% (39/42)	81.0-97.5	89.8% (176/196)	84.8-93.3
	Retest	198	88.4% (137/155)	82.4-92.5	90.7% (39/43)	78.4-96.3	88.9% (176/198)	83.8-92.5
ERBB2/HER2 Xpert vs. IHC	Original	180	100% (22/22)	85.1-100	92.4% (146/158)	87.2-95.6	93.3% (168/180)	88.7-96.1
	Retest	180	100% (22/22)	85.1-100	92.4% (146/158)	87.2-95.6	93.3% (168/180)	88.7-96.1
ERBB2/HER2 Xpert vs. FISH	Original	178	100% (28/28)	87.9-100	92.0% (138/150)	86.5-95.4	93.3% (166/178)	88.6-96.1
	Retest	178	100% (28/28)	87.9-100	92.0% (138/150)	86.5-95.4	93.3% (166/178)	88.6-96.1
ERBB2/HER2 Xpert vs. IHC +FISH	Original	197	100% (27/27)	87.5-100	91.2% (155/170)	86.0-94.6	92.4% (182/197)	87.8-95.3
	Retest	197	100% (27/27)	87.5-100	91.2% (155/170)	86.0-94.6	92.4% (182/197)	87.8-95.3

Comparison	Data Set[a]	Total (n)[b]	PPA	95% CI	NPA	95% CI	OPA	95% CI
MKi67/Ki67 Xpert vs. IHC	Original	148	88.7% (110/124)	81.9-93.2	100% (24/24)	86.2-100	90.5% (134/148)	84.7-94.3
	Retest	151	88.8% (111/125)	82.1-93.2	100% (26/26)	87.1-100	90.7% (137/151)	85.0-94.4

[a] Original = 1X lysate according to instructions in the instructions for use; Retest = retest result on a 4X concentrated lysate in cases where the original specimen (1X lysate) gave an indeterminate result for PGR and/or MKi67.

[b] Specimens with non-determinate or indeterminate Xpert results, specimens with equivocal or intermediate IHC results, specimens with failed IHC and failed FISH are excluded.

19 Analytical Performance

19.1 Analytical Sensitivity/Minimum Assay Input

Minimum assay input was determined by assessing the maximum CYFIP1 Ct (reference gene) that accurately determines the sample input needed for robust assay performance. This sample input ensures valid results are obtained in most clinical FFPE samples tested. Samples with a CYFIP1 Ct value greater than that allowed will generate an **INVALID** result.

The analytical sensitivity/minimum assay input for the Xpert Breast Cancer STRAT4 test, defined as the maximum CYFIP1 Ct that results in ≥95% valid results, was established using dilutions of FFPE clinical sample lysates to challenge the CYFIP1 Ct. To assess the sensitivity of the CYFIP1 Ct, a FFPE clinical sample lysate was serially diluted and tested with N=20 replicates per dilution level across 3 days until ≤95% of test results were valid. The dilution levels included one specimen at the expected minimum assay input, two levels below that and two levels above. Testing was performed on two lots of Xpert Breast Cancer STRAT4 cartridges.

Prior to the initiation of the study, limit of blank testing was performed with N=60 replicates using two independent lots of Xpert Breast Cancer STRAT4 cartridges. The limit of blank sample consisted of a blank paraffin section (no tissue sample), and all test results showed expected **INVALID** result calls. Serial dilutions of the clinical FFPE tissue sample input at 1/1000 yielded 20/20 valid CYFIP1 Cts with mean Ct = 33.4 and 0.6 SD from lot 1 of the Xpert Breast Cancer STRAT4 test and mean Ct = 33.6 and 0.5 SD from lot 2. Further dilutions with later CYFIP1 Ct values failed to meet the ≥95% valid results required for the study. Table 4 summarizes the number of valid test runs at each serially diluted sample input level as Relative Dilution or as Mean CYFIP1 Ct. The Analytical Sensitivity using two lots of Xpert Breast Cancer STRAT4 test cartridges demonstrated minimum assay input requirement for CYFIP1 Ct = 33.4. This value, combined with assay variability would allow the upper CYFIP1 Ct = 35 limit to be set for the Xpert Breast Cancer STRAT4 test.

Table 4. Minimum Assay Input in Xpert Breast Cancer STRAT4

Kit Lot	Sample Input (Relative Dilution)	Mean CYFIP1 Ct	SD	N Valid Run (Ct ≤ 35)
00801 (Lot 1)	1/20	27.6	0.4	20/20
	1/100	29.8	0.3	20/20
	1/1000	33.4	0.6	20/20
	1/2000	34.2	0.5	9/20
	1/4000	34.5	0.5	2/20
	NTC	n/a	n/a	0/20
00903 (Lot 2)	1/20	27.8	0.3	20/20
	1/100	30.0	0.3	20/20
	1/1000	33.6	0.5	20/20
	1/2000	34.2	0.4	9/20
	1/4000	34.6	0.0	1/20
	NTC	n/a	n/a	0/20

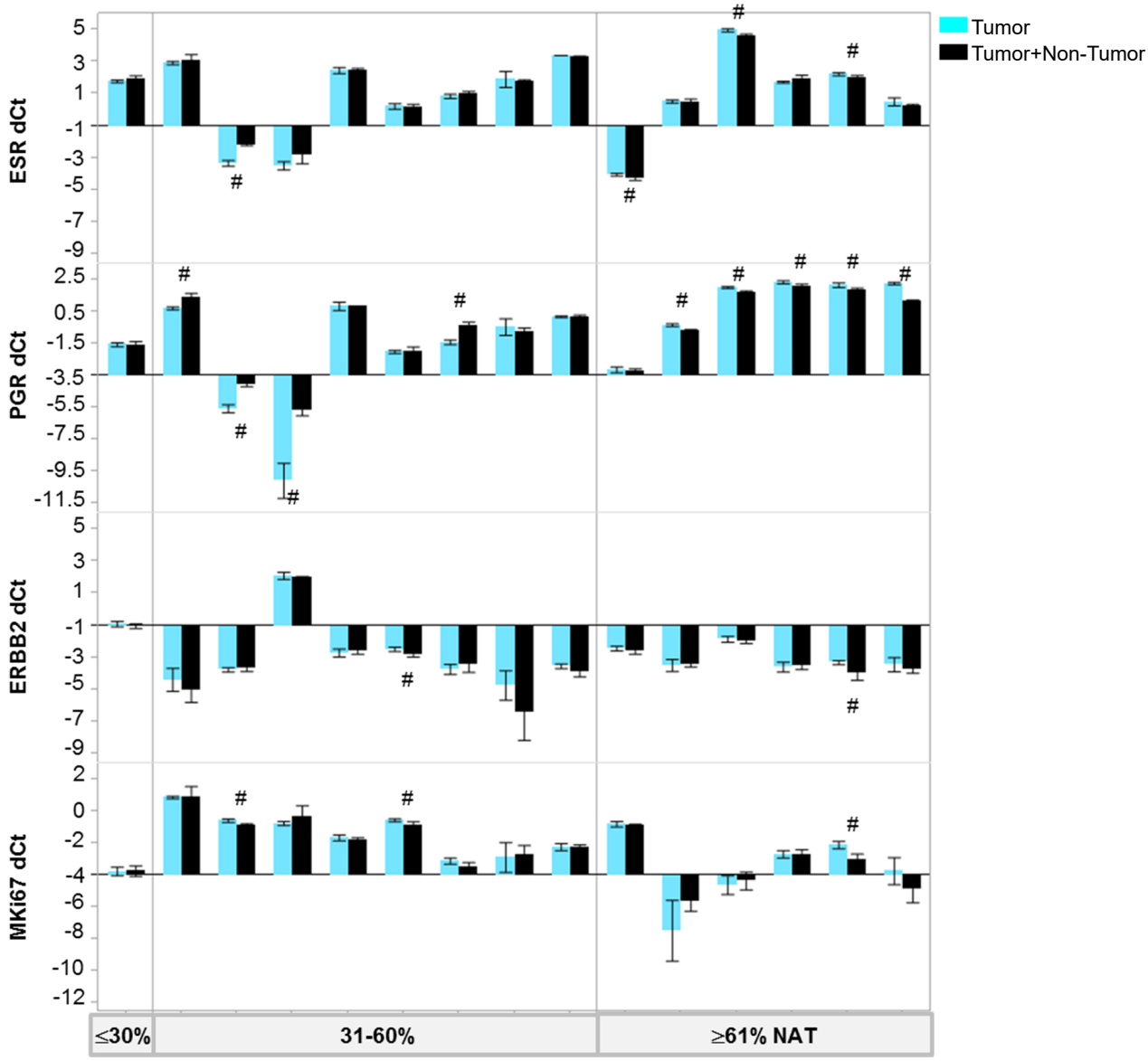
19.2 Interference Testing

Adjacent Normal/Non-Tumor Tissue

Normal Adjacent (non-tumor) Tissues (NAT) are commonly present among breast cancer tissue specimens as contaminants that potentially interfere with specific target detection. The Xpert Breast Cancer STRAT4 test may require a pathologically verified breast tumor FFPE section to be macrodissected to minimize potential effects of non-tumor contaminants in applicable cases as determined by a pathologist. To assess the effect of adjacent normal/non-tumor tissues, fifteen (15) FFPE tissue blocks with invasive breast carcinoma containing 21-98% surrounding NAT were tested with the Xpert Breast Cancer STRAT4 test with and without macrodissection. Xpert Breast Cancer STRAT4 testing was performed with N=4 replicates from the same lysate per condition. ESR1, PGR, ERBB2, and MKi67 dCts for each tissue sample with macrodissection (bar graph in blue) or without macrodissection (bar graph in black) were first evaluated via One-Way ANOVA to determine statistical interference of NAT. Clinically significant interference by NAT was considered present when the ddCt (delta-delta Ct) between macro and non-macrodissected samples was >1.0 and there was a change to the test result. The study results are summarized in Figure 6.

ESR1, PGR, ERBB2, and MKi67 dCts of all 15 samples were grouped based on % NAT (≤30%, 31-60%, or ≥61%). Blue and black vertical bar graphs with SD represent mean target dCts from N=4 replicates of macro- and non-macrodissected FFPE sections of a FFPE invasive breast cancer block. All 15 FFPE blocks (N=1 below 30% NAT, N=8 with 31-60% NAT, and N=6 above 60% NAT) showed either no statistical significance of the adjacent normal/non-tumor tissue interference based on One-Way ANOVA analyses with p-value ≥0.05; or no clinical significance (marked as #) if the variation in delta Ct values of each target between macrodissected or non-macrodissected samples was ≤1.0 or when the target test results (positive, negative) remained unaffected.

Figure 6. Adjacent Normal/Non-Tumor Tissue Interference to the Xpert Breast Cancer STRAT4 Target dCts



DCIS, Necrotic, Hemorrhagic Tissue

To assess the effect of ductal carcinoma in situ (DCIS), necrotic, and hemorrhagic tissues, a total of 9 FFPE breast tumor samples (3 FFPE breast tumor blocks containing 3-61% DCIS, 3 FFPE blocks containing 10-65% necrotic tissue, and 3 FFPE blocks containing 15-41% hemorrhagic tissue) were tested with the Xpert Breast Cancer STRAT4 test with and without macrodissection. Xpert Breast Cancer STRAT4 test was performed with N=4 replicates from the same lysate per condition. All test conditions were found to have either no statistical or no clinically significant impact from varying DCIS, necrosis, and hemorrhagic tissue contaminations using the Xpert Breast Cancer STRAT4 test (graphical data not shown).

Human Genomic DNA (hgDNA)

The Xpert Breast Cancer STRAT4 test utilizes highly specific primers and probes to efficiently hybridize with the target ESR1, PGR, ERBB2, and MKi67 mRNA templates from a pool of genomic nucleic acids (human genomic DNA = hgDNA). To assess the effect of hgDNA on the Xpert Breast Cancer STRAT4 test, 10 FFPE breast tumor blocks with varying invasive ductal carcinoma cell content were macrodissected and tested with and without addition of 25 ng of hgDNA to the FFPE sample lysates using the Xpert Breast Cancer STRAT4 test in N=4 replicates from the same lysate per condition. All test conditions were found to have either no statistical or no clinically significant impact of the hgDNA interference (graphical data not shown).

19.3 Carry-Over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges minimize carry-over contamination from very high positive samples into subsequent negative samples run in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately following a high ESR1/PGR/ERBB2/MKi67 positive sample. The negative sample consisted of *in vitro*-transcribed (IVT) RNA containing CYFIP1 transcript at 5 x 10^4 copies to ensure presence of a reference gene target. The high positive sample consisted of IVT RNA containing CYFIP1 transcript at 5 x 10^5 copies, and IVT RNA containing ESR1, PGR, ERBB2 and MKi67 transcripts at 5 x 10^6 copies, prepared as FFPE lysate. The testing scheme was repeated 41 times using a single GeneXpert module for a total of 20 high positive and 21 negative samples. All 20 high positive samples were correctly reported as ESR1/PGR/ERBB2/MKi67 POSITIVE and all 21 negative samples were correctly reported as ESR1/PGR/ERBB2/MKi67 NEGATIVE.

19.4 Assay Reproducibility and Precision

Reproducibility of Xpert Breast Cancer STRAT4 was evaluated using a panel of five lysate specimen samples.

Three panel members were prepared by adding *in vitro* transcript (IVT) RNA into FFPE lysis buffer spiked within ~2dCts of the dCt cut-offs for ESR1 (1 IVT RNA), PGR (2 IVT RNA), and ERBB2 (3 IVT RNA), and having CYFIP1 Ct values ~2-3 Cts from the Minimum Assay Input level.

Two panel members (4 Clinical FFPE Sample and 5 Clinical FFPE Sample) were created from pooled clinical FFPE samples in FFPE lysis buffer to generate CYFIP1 Ct values near the Minimum Assay Input and to have dCt cut-off values for all targets across the reportable range and, to the extent possible, near the assay dCt cut-offs.

Two operators at each of the three study sites tested two panels of five samples per day over six testing days (five samples x six days x two operators x two replicates x three sites). A total of 72 replicates per sample were tested. Three lots of Xpert Breast Cancer STRAT4 cartridges were used at each of the three testing sites. The Xpert Breast Cancer STRAT4 test was performed according to the procedure in this instruction for use.

The reproducibility of the Xpert Breast Cancer STRAT4 was evaluated in terms of the dCt for each of the four targets for each panel. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-lots, between-days, between-operators, and within-assays for each panel member are presented in Table 5.

Table 5. Summary of Reproducibility Data

Sample	Assay Channel (Analyte)	N[a]	Mean dCt	Between-Site		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
				Var	CV (%)	Var	CV (%)	Var	CV (%)	Var	CV (%)	Var	CV (%)	Var	CV (%)
1-IVT RNA	ESR1	72	0.20	0.00	0.00	0.03	29.30	0.00	0.00	0.00	1.80	0.07	68.90	0.11	0.33
	PGR	72	-0.03	0.00	0.00	0.01	14.70	0.00	2.30	0.00	0.00	0.06	83.00	0.07	0.26
	ERBB2	72	-2.42	0.00	0.00	0.04	27.90	0.02	11.40	0.00	2.60	0.08	58.10	0.13	0.36
	MKi67	70	-2.55	0.00	0.00	0.32	62.10	0.00	0.00	0.00	0.00	0.20	37.90	0.52	0.72
2-IVT RNA	ESR1	72	-1.03	0.00	1.60	0.01	9.20	0.01	5.50	0.00	0.00	0.10	83.70	0.12	0.35
	PGR	72	-1.26	0.00	0.00	0.01	12.20	0.00	0.00	0.01	10.70	0.04	77.10	0.05	0.23
	ERBB2	72	-3.49	0.01	4.80	0.03	31.60	0.00	0.00	0.00	0.40	0.07	63.20	0.11	0.33
	MKi67	72	-3.53	0.00	0.00	0.08	49.00	0.00	0.00	0.00	0.00	0.08	51.00	0.16	0.40
3-IVT RNA	ESR1	72	3.64	0.00	0.00	0.01	8.40	0.01	16.50	0.00	0.00	0.06	75.10	0.08	0.29
	PGR	72	3.34	0.00	3.40	0.00	0.00	0.01	9.70	0.00	5.40	0.05	81.50	0.06	0.25
	ERBB2	72	0.91	0.02	20.60	0.01	10.30	0.00	0.00	0.00	0.00	0.05	69.10	0.08	0.28
	MKi67	72	1.14	0.00	0.00	0.02	15.40	0.02	18.00	0.00	0.00	0.07	66.60	0.10	0.31
4-FFPE Clinical Sample	ESR1	72	-0.11	0.00	0.00	0.00	0.00	0.00	14.40	0.00	15.90	0.02	69.70	0.03	0.17
	PGR	72	-1.99	0.00	6.30	0.01	19.70	0.00	2.50	0.00	0.00	0.02	71.60	0.03	0.18
	ERBB2	72	-2.39	0.02	31.30	0.00	2.20	0.00	0.00	0.00	3.70	0.05	62.80	0.07	0.27
	MKi67	72	-0.93	0.00	0.00	0.02	36.50	0.00	0.00	0.00	0.00	0.03	63.50	0.04	0.21

Sample	Assay Channel (Analyte)	N[a]	Mean dCt	Between-Site		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
				Var	CV (%)	Var	CV (%)	Var	CV (%)	Var	CV (%)	Var	CV (%)	Var	CV (%)
5-FFPE Clinical Sample	ESR1	72	-2.83	0.00	0.00	0.05	13.70	0.00	0.00	0.00	0.00	0.34	86.30	0.39	0.63
	PGR	72	-5.66	0.00	0.00	0.02	3.60	0.03	4.40	0.00	0.00	0.56	92.00	0.60	0.78
	ERBB2	72	1.93	0.00	2.90	0.00	3.00	0.00	0.00	0.00	0.00	0.03	94.20	0.03	0.17
	MKi67	72	-1.57	0.00	1.70	0.01	17.10	0.01	9.00	0.00	11.10	0.05	61.10	0.09	0.29

[a] Results with valid delta Ct values out of 72

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22 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
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- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

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23 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
CE	CE marking – European Conformity
EC REP	Authorized Representative in the European Community
	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
CH REP	Authorized Representative in Switzerland
	Importer

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24 Revision History

Revision	Description of Change
Throughout	Deleted ONCore software references.