
Cepheid
A better way.
Assay Training:
Xpert® Breast Cancer STRAT4
Technical Training for CE-IVD product only
1 © Cepheid
301-8042, Rev. A May 2017
CE-IVD. For in vitro diagnostic use.

Training Agenda

- Xpert Breast Cancer STRAT4 Training
  - Clinical Application
  - Reagents
  - Kit storage and handling
  - Precautions
  - Preparing sample
  - Preparing cartridge
  - Quality Control
  - Results analysis

© Cepheid
CE-IVD. For *in vitro* diagnostic use.

Cepheid

- During the presentation, we will study and discuss:
- Clinical use of Xpert Assay Name
- Reagents provided/required for this Assay Name
- Proper sample collection and storage
- Kit handling and storage
- Preparation of Xpert Assay cartridge

- We will also cover:
- How to read results and the different result you can get
- What are the features included in this Assay for ensuring quality assured results

- At the end, we will discuss your concern and provide you with better explanation

Cepheid
A better way.

Training Objectives

At the end of the training, user will be able to:

- Store and handle the Xpert Breast Cancer STRAT4 cartridge kit
- Store and handle the Xpert FFPE Lysis kit
- Follow proper laboratory safety precautions
- Process and transport specimens
- Perform the cartridge set up and run the assay
- Report the various software-generated results
- Understand the assay control strategy

 © Cepheid CE-IVD. For *in vitro* diagnostic use. Cepheid.

- Once we will complete this training, you all will be able to :
  - Properly store and handle the Xpert® Assay cartridge kit
  - Follow proper laboratory safety precautions
  - Collect and transport appropriate specimen
  - Prepare a cartridge and run the Breast Cancer assay
  - Report the various software generated results
  - Understand the Assay control strategy

Cepheid. A better way.

Cepheid.
A better way.

Breast Cancer Facts:

- Breast cancer is the one of the most common cancers among women worldwide.
- It is the most common cause of cancer mortality among women in developing countries.
- It is the second most common cause of cancer mortality (after lung cancer) among women in developed countries.
- Breast cancer mortality has decreased by 34% since 1990 largely due to improved treatment and early detection.

References
American Cancer Society, Cancer Facts and Figures 2015. Atlanta, GA: American Cancer Society, 2015.
International Agency for Research on Cancer (IARC) and World Health Organization (WHO). GLOBOCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide in 2012 http://globocan.iarc.fr/old/FactSheets/cancers/breast-new.asp.
American Cancer Society, Breast Cancer Facts and Figures 2013-2014, Atlanta, GA: American Cancer Society, 2013.

 © Cepheid CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid.
A better way.

What is Breast Cancer?

- Breast Cancer primarily occurs in ducts & lobules of the breast
- Breast cancer can be categorized into four intrinsic molecular subtypes that provide prognostic and predictive information
  - Luminal A, Luminal B, HER2-enriched & Triple Negative (Basal)
- Selection of first therapeutic regimen determined by the expression of four biomarkers
  - Estrogen Receptor (ER [protein] or ESR1 [mRNA]), Progesterone Receptor (PR [protein] or PGR [mRNA]), Human Epidermal Growth Factor Receptor (HER2 [protein] or ERBB2 [mRNA]), Marker of Proliferation Ki-67 (Ki67 [protein] or MKi67 [mRNA])
  - Standard practice for initial characterization of every breast cancer patient
  - Biomarkers are both Prognostic and Predictive
- Current methods for biomarker measurement include:
  - Immunohistochemistry (IHC) to assess protein expression
  - Fluorescence in situ Hybridization (FISH) to look for gene amplification (HER2 only)

References

de Matos LL, Trufelli DC, Luongo de Matos MG, da Silva Pinhal MA. Immunohistochemistry as an Important Tool in Biomarkers Detection and Clinical Practice. Biomarker Insights 2010:5, 9-20.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid.
A better way.

The Need:

- Faster Result TAT
  - Current turn around time typically takes days. Shortening wait time to treatment initiation for women newly diagnosed with breast cancer is an advantage for the GX.
- More Standardized, Less Subjective Procedure that is much easier to perform
  - Immunohistochemistry (IHC) and Fluorescence *in situ* hybridization (FISH) staining can be subjective and difficult to standardize across laboratories. For IHC, not all antibodies are created equal.
- Cheaper Alternative
  - Staining equipment and reagents can be expensive
- Improvement in Patient Management Pathway
  - Allow laboratory testing to be performed closer to the patient and get faster results
  - Allow pathologists to control aspects of testing, while limiting the need for reference laboratory testing
  - Likely represents the only workable solution for patients in the developing world where IHC very difficult and expensive to perform. FISH is not available at all for developing world patients.

© Cepheid
CE-IVD. For *in vitro* diagnostic use.
Cepheid.
Cepheid. A better way.

The Solution:

- Empowers the pathologist
  - Lab workforce optimization - Highly reproducible, easy to use, & easy to train
  - Assay can be performed in histology or clinical lab
- Reduced subjectivity when compared to IHC
  - Excellent concordance with recognized standards – 90% overall concordance across all 4 markers.
  - Continuous output with no equivocal results
  - Easy to interpret results
- Potential workflow improvements
  - Actionable results – Fast TAT in ~2 hours
  - True random access
- Simplified lysis procedure for FFPE
  - A conveniently packaged and easy to use kit
- Commercially available external controls*

© Cepheid
CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid.
A better way.

The Cepheid Solution

- Four mRNA targets measured (ESR1, PGR, ERBB2, and MKi67).
- Cytoplasmic FMR1 interacting protein 1 (CYFIP1) reference gene functions as the Sample Adequacy Control and is used to normalize the mRNA expression levels.
- Simple and easy to use
- Closed cartridge system minimizes risk of contamination
- 3 internal controls for each sample
  - Probe Check control (PCC)
  - Cepheid Internal Control (CIC)
  - Sample Adequacy Control (SAC)

© Cepheid
CE-IVD. For *in vitro* diagnostic use.

Cepheid
A better way.

Cepheid.
A better way.

Intended Use*

Xpert Breast Cancer STRAT4 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), 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 Assay 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 assay 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.

*CE-IVD. In Vitro Medical Device.

© Cepheid

Cepheid

Cepheid
A better way.

System and Reagent Requirements

GeneXpert Systems

- 6-color GeneXpert instrument
- GeneXpert software version 4.7b or higher
- Xpertise software version 6.4b or higher

Test Kits (CE-IVD)

- GXBCSTRAT4-CE-10

Materials Required but not Provided

- Xpert® FFPE Lysis Kit, Catalogue #GXFFPE-LYSIS-CE-10
- ≥95% Ethanol
- Vortex Mixer
- Pipettes and aerosol filter pipette tips to pipette 5, 20, 260, 520 and 1200µL
- 80°C Heat Block to hold 1.5mL microcentrifuge tubes
- Microcentrifuge to spin 1.5mL microcentrifuge tubes

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

Xpert Breast Cancer STRAT4 Kit Components

	Xpert Breast Cancer STRAT4
Catalog Number	**GXBCSTRAT4-CE-10**
Tests per kit	10
Contents per test cartridge	Reagent beads
	Elution and Rinse Reagents
Kit CD	Assay Definition File (ADF)- .gxa
	Assay Import Instructions
	Package Insert (PDF)
	ONCore Software report file - .onc
Storage	2-28°C

Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

13 © Cepheid CE-IVD. For *in vitro* diagnostic use. Cepheid.

- Although all necessary reagents/materials are included in the kit, however some additional materials you will need.
- System requirement: GeneXpert system with GeneXpert software version xx or higher - Xpertise version xx or higher (the lower versions would not be able to run the Ultra test)
- Test kit – kit of xx or xx configuration

  Sample Container: Leak proof, sterile screw-capped collection containers

- Optional:
- Personal Protective Equipment (PPE).
- 1: 10 diluted Chlorine Bleach
- 70% ethanol to clean and disinfect the modules during the maintenance procedure

  - The use of a UPS with Surge Protector is strongly recommended (Real-Time PCR implies that even a very short power interruption will stop the run)
  - A Printer may be used to print the result reports

Cepheid. A better way.

– Vortex is optional for sample preparation steps

Xpert FFPE Lysis Kit Components

	Xpert FFPE Lysis Kit
Catalog Number	**GXFFPE-LYSIS-CE-10**
Tests per kit	10
Lysis Tubes (1.5mL)	10
Sample Vials (5mL)	10
FFPE Lysis Reagent	13mL
Proteinase K (PK)	250µL
Storage	2-28°C

Kits contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

14 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

Good Laboratory Practice

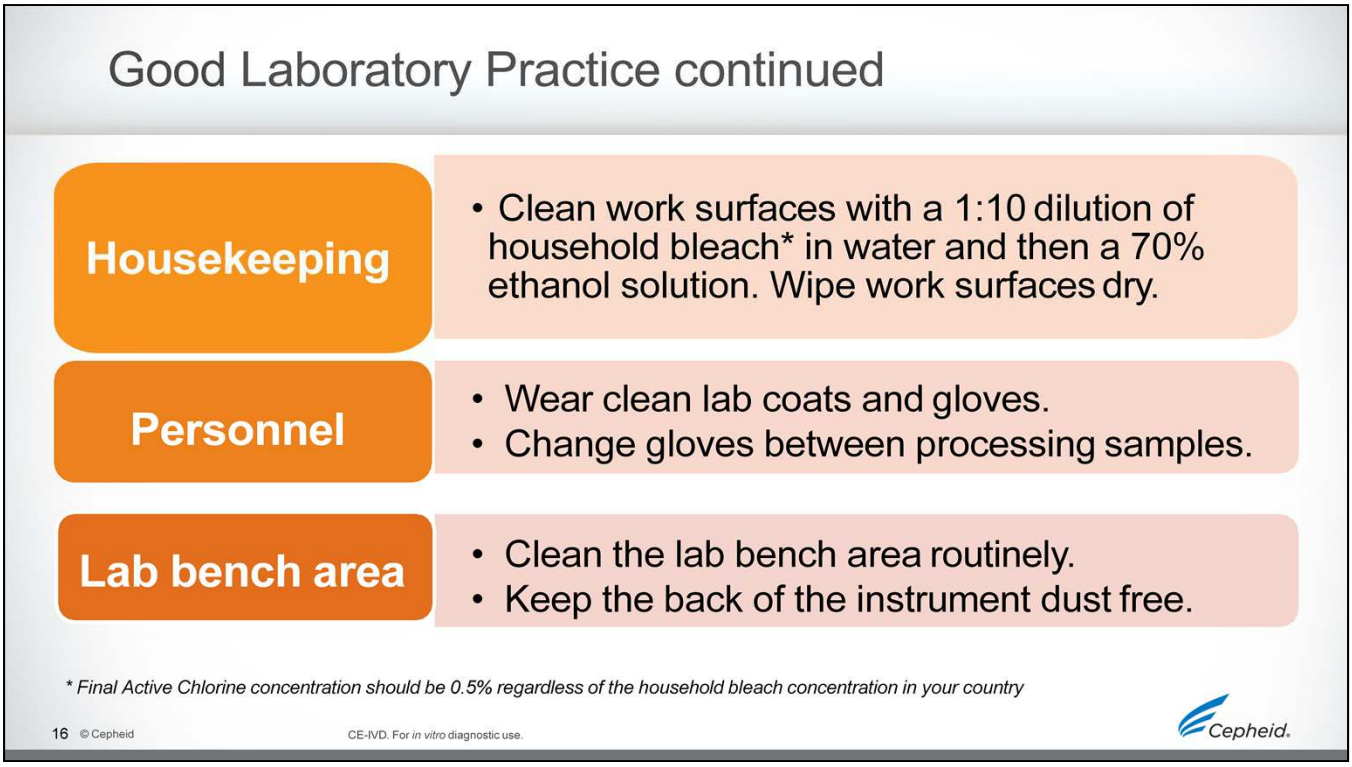
PCR laboratory setup	• Cartridge/reagent preparation → Sample addition → Detection
Specimen and reagent storage	• Store specimens separately from reagents to prevent reagent contamination.
Equipment	• Use filtered pipette tips, when needed. • Follow the manufacturer's recommendation for calibration and maintenance of the lab equipment.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

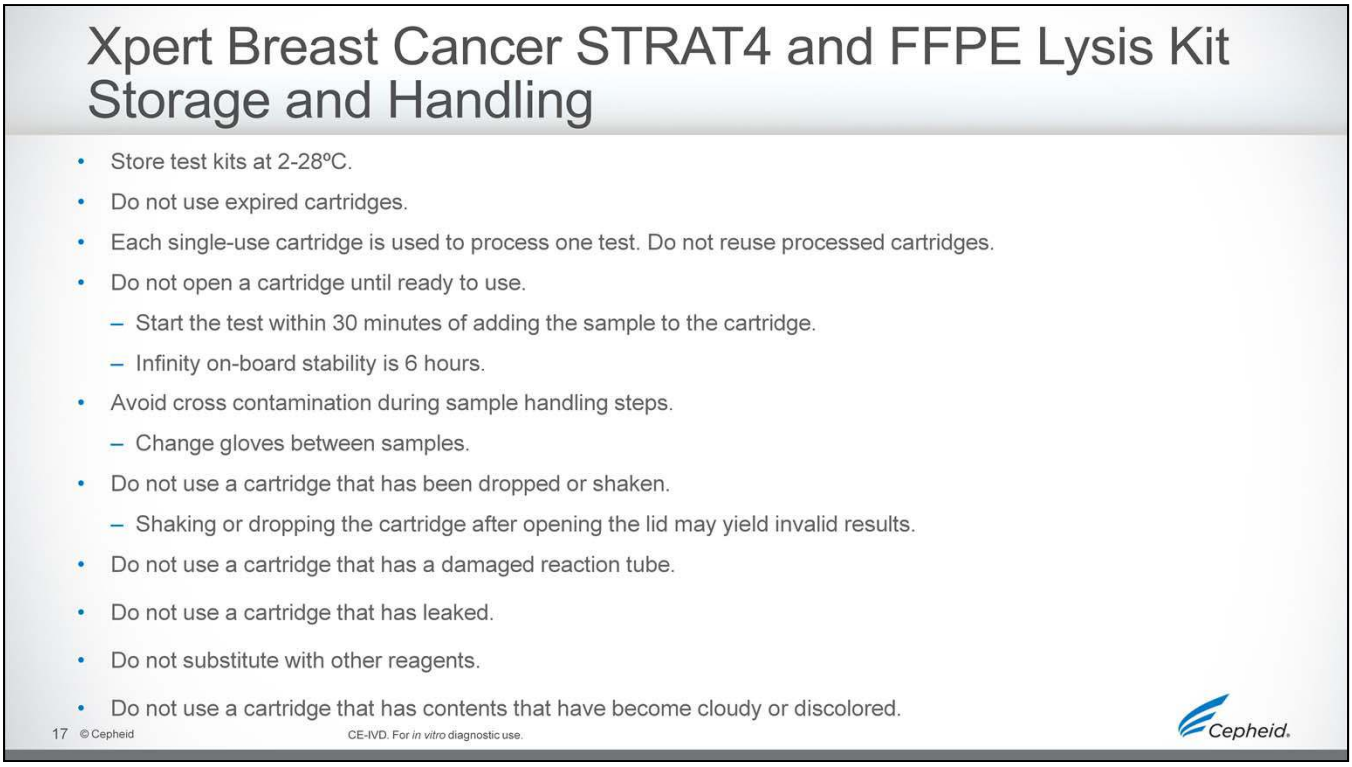
Cepheid
A better way.



- Though GeneXpert does not need strict molecular biology laboratory setup and practices, it is important to follow Good Laboratory Practice per training to Molecular Biology laboratories

Ensure that the equipment is controlled, such as the pipettes and vortex, etc.

Please consult WHO or CDC bio-safety manual for detail safety procedures.



As for any biological products, you must take some precautions while handling it, to preserve the kit integrity and functionality:

- Store the Xpert Assay cartridges and reagents at 2–28°C
- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use Collection Reagent tubes that have not been validated by Cepheid
- Open the Assay cartridge lid only when adding the Sample, close the lid and proceed with the next one

Cepheid.
A better way.

Warnings and Precautions:

- Handle all sample and kit reagents using appropriate techniques to prevent or minimize RNase and/or DNase contamination.
- Do not reuse macrodissection blades, pipette tips, or tubes/vials to avoid cross contamination during sample handling.
- Incomplete removal (scraping) of the 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.
- Treat all biological samples, including used cartridges, with standard precautions.
- FFPE tissue must be treated with Xpert FFPE Lysis Kit.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.

18 © Cepheid CE-IVD. For *in vitro* diagnostic use. Cepheid.

- **Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. PG 146:** BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Liquifaction and concentration of sputa for acid-fast staining may be conducted safely on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before processing.

- Do not shake the cartridge
- Do not use a cartridge that… :
  - appears wet, has leaked or if the lid seal appears to have been broken
  - appears damaged
  - has been dropped after removing it from packaging
  - has been dropped or shaken after adding the sample to it

Cepheid. A better way.

- has a damaged reaction tube
- has been used: each cartridge is single-use to process one test is expired
- Do not reuse spent disposable pipettes

- Unused cartridges may be discarded as Chemical waste and used cartridges as infectious biological waste. Sample reagent bottles should be considered as Chemical waste

Cepheid.
A better way.

FFPE Tissue Requirements

- Unstained FFPE tissue sections or tissue scrolls may be used.
- Follow ASCO/CAP guidelines for preparing FFPE tissue.
- Specimens must have been fixed in only 10% Neutral Buffered Formalin (NBF) for 6 - 72 hours.
- FFPE lysate should be prepared from the FFPE tumor block with the greatest area of viable breast carcinoma (a minimum of 30% tumor cellularity).
- If macrodissection is required, use an adjacent H&E stained slide from the FFPE tumor block as a guide to mark the unstained slide.
- 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 to achieve valid results.
- Some FFPE tissues greater than 10 years old may be of insufficient quality for GeneXpert analysis (RNA degrades over time, even in FFPE tissues).

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

Slide/Scroll Preparation Continued

1. Cut two tissue sections and place each on a separate glass slide.
2. Stain one slide with H&E.
3. Pathologist examines the H&E slide to demarcate tumor and non-tumor area(s).
4. If appropriate tumor area(s) is present (>30% and no DCIS), the entire section from the second slide may be used. Alternatively, it can be macrodissected to remove specific areas for testing.

FFPE tissue

Image by Thomas Sherwood

Image by Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

Scraping Instructions

Label a 1.5 mL lysis tube (provided) for each sample to be processed.

- If macrodissection is not required:
  - Using a new razor blade or scalpel for each tissue sample to be processed, completely remove (scrape) the invasive tumor tissue section from the slide and transfer to the labeled 1.5 mL lysis tube.
- If macrodissection is required:
  - Examine the H&E stained slide (pathologist).
  - Identify (and outline) the tumor area to be selected for testing. Refer to Xpert assay package insert for required number of slides or minimum tumor cellularity requirement.
  - Outline the tumor area to be used for the assay on the backside of the unstained slide(s) by aligning it with the corresponding H&E stained slide and transposing the outlined area.
  - Perform macrodissection (pathologist or technician).
  - Using a new razor blade or scalpel for each tissue sample to be processed, completely remove (scrape) the outlined invasive tumor tissue from the slide (see Figure 1) and transfer to the labeled 1.5 mL lysis tube.

Figure 1: Examples of proper (recommended) and improper tissue (not recommended) removal from slide

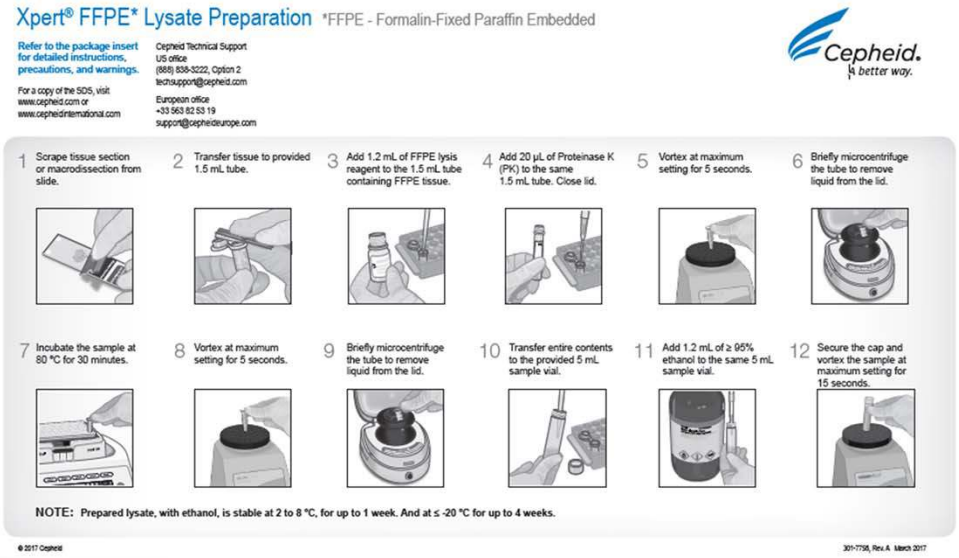
Images by Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid.
A better way.

Xpert FFPE Lysate Preparation Card



Xpert® FFPE* Lysate Preparation *FFPE - Formalin-Fixed Paraffin Embedded

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support
US office
(888) 838-3222, Option 2
techsupport@cepheid.com

European office
+33 563 82 53 19
support@cepheideurope.com

Cepheid. A better way.

1 Scrape tissue section or macrodissection from slide.

2 Transfer tissue to provided 1.5 mL tube.

3 Add 1.2 mL of FFPE lysis reagent to the 1.5 mL tube containing FFPE tissue.

4 Add 20 µL of Proteinase K (PK) to the same 1.5 mL tube. Close lid.

5 Vortex at maximum setting for 5 seconds.

6 Briefly microcentrifuge the tube to remove liquid from the lid.

7 Incubate the sample at 80 °C for 30 minutes.

8 Vortex at maximum setting for 5 seconds.

9 Briefly microcentrifuge the tube to remove liquid from the lid.

10 Transfer entire contents to the provided 5 mL sample vial.

11 Add 1.2 mL of ≥ 95% ethanol to the same 5 mL sample vial.

12 Secure the cap and vortex the sample at maximum setting for 15 seconds.

NOTE: Prepared lysate, with ethanol, is stable at 2 to 8 °C, for up to 1 week. And at ≤ -20 °C for up to 4 weeks.

© 2017 Cepheid

301-7758, Rev. A March 2017

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

- Read the protocol from Card as such. Prior understanding of Protocol will help trainer to explain the card in a smooth way.

FFPE Tissue Processing

1. Preheat heat block to 80°C.
2. Add 1.2 mL of FFPE lysis reagent to the 1.5 mL tube containing FFPE section/scroll.
3. Add 20 µL of Proteinase K (PK) to the same 1.5 mL lysis tube.
4. Close lid.
5. Vortex the sample continuously at a maximum setting for 5 seconds.
6. Briefly microcentrifuge the tube to remove liquid from the lid.
7. Incubate 1.5 mL lysis tube containing sample and lysis reagent in 80°C heat block for 30 minutes.
8. Vortex the sample continuously at maximum setting for 5 seconds.
9. Briefly microcentrifuge the tube to remove liquid from the lid.
10. Using a pipette, transfer the entire contents (~1.2 mL) to a provided 5 mL sample vial.
11. Label the vial for each sample to be processed.
12. Add 1.2 mL of ≥ 95% ethanol to the same 5 mL sample vial.
13. Secure the cap and vortex the sample continuously at maximum setting for 15 seconds.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

Xpert Breast Cancer STRAT4 Cartridge Prep Card

Xpert® Breast Cancer STRAT4 Cartridge Preparation

Cepheid. A better way.

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support
US office
(888) 838-3222, Option 2
techsupport@cepheid.com
European office
+33 563 82 53 19
support@cepheideurope.com

NOTE: If the preparation of the FFPE lysate, as described on the reverse of this card, was just completed, Step 2 of the instructions below may be skipped.

1 Obtain one cartridge and one prepared FFPE lysate for each sample to be tested.

2 Vortex FFPE lysate for 15 seconds.

3 Open the Xpert cartridge lid.

4 Pipet 520 µl of prepared lysate from the 5 mL vial and transfer into the sample chamber.

5 Close the Xpert cartridge lid.

6 Start the assay within the timeframe specified in the package insert.

© 2017 Cepheid

301-7798, Rev. A. March 2017

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

Assay Procedure – Cartridge Preparation

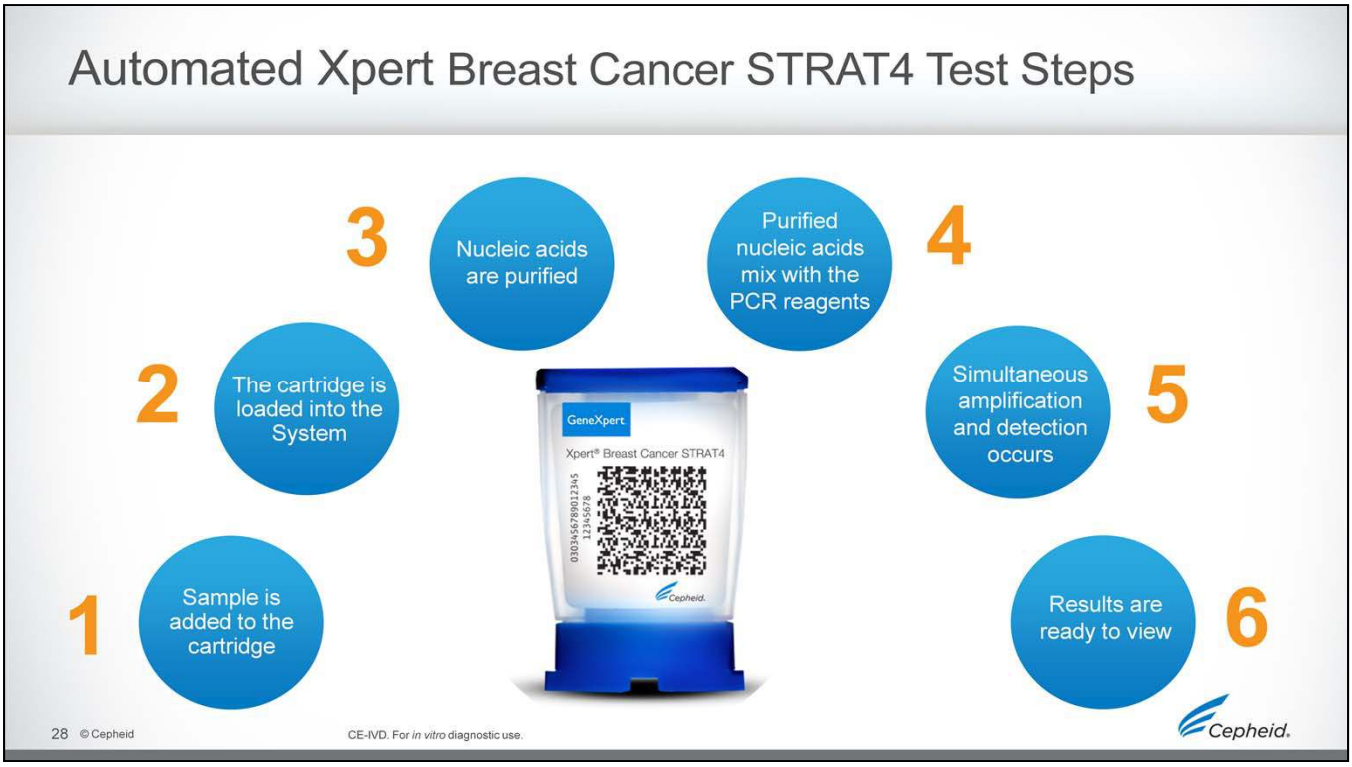
If using a GeneXpert Dx instrument	• Start the test within 30 minutes of adding the prepared sample to the cartridge.
If using a GeneXpert Infinity instrument	• Order the test and put the cartridge on the conveyor within 30 minutes of adding the prepared sample to the cartridge. • Remaining on-board stability is tracked by the Xpertise® Software so that tests are run prior to the 6 hour on-board expiration.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid.
A better way.



- Here is a general protocol for cartridge operation
  - Sample is added to the cartridge
  - Cartridge is loaded into the System
  - Nucleic acids are purified
  - Purified nucleic acids mix with the PCR reagents
  - Real time PCR occurs
  - Results are ready to be viewed

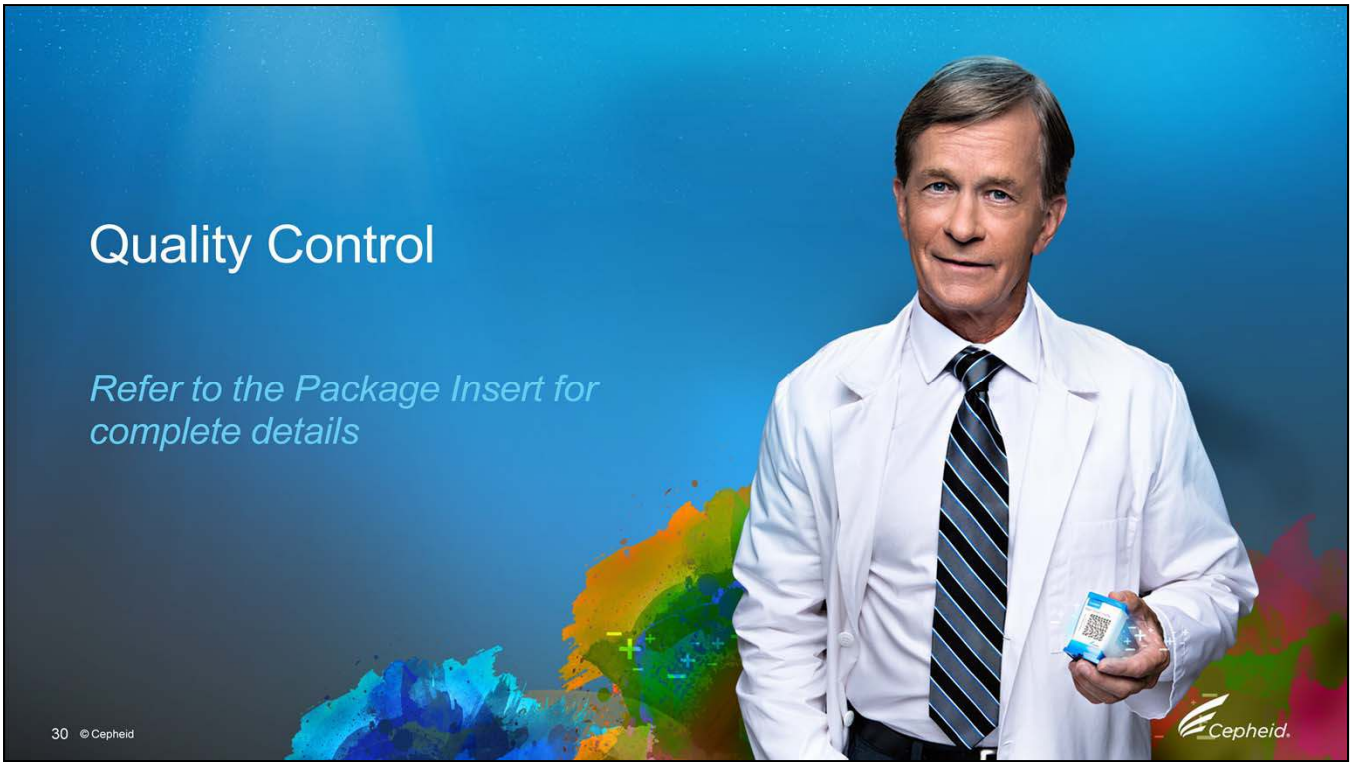
Cepheid.
A better way.

Waste Disposal

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions.
- Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.
- If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

 © Cepheid CE-IVD. For *in vitro* diagnostic use.

- The commercial controls are available. Please follow your institution guidelines for frequency of QC.



Cepheid.
A better way.

Instrument System Control – Check Status

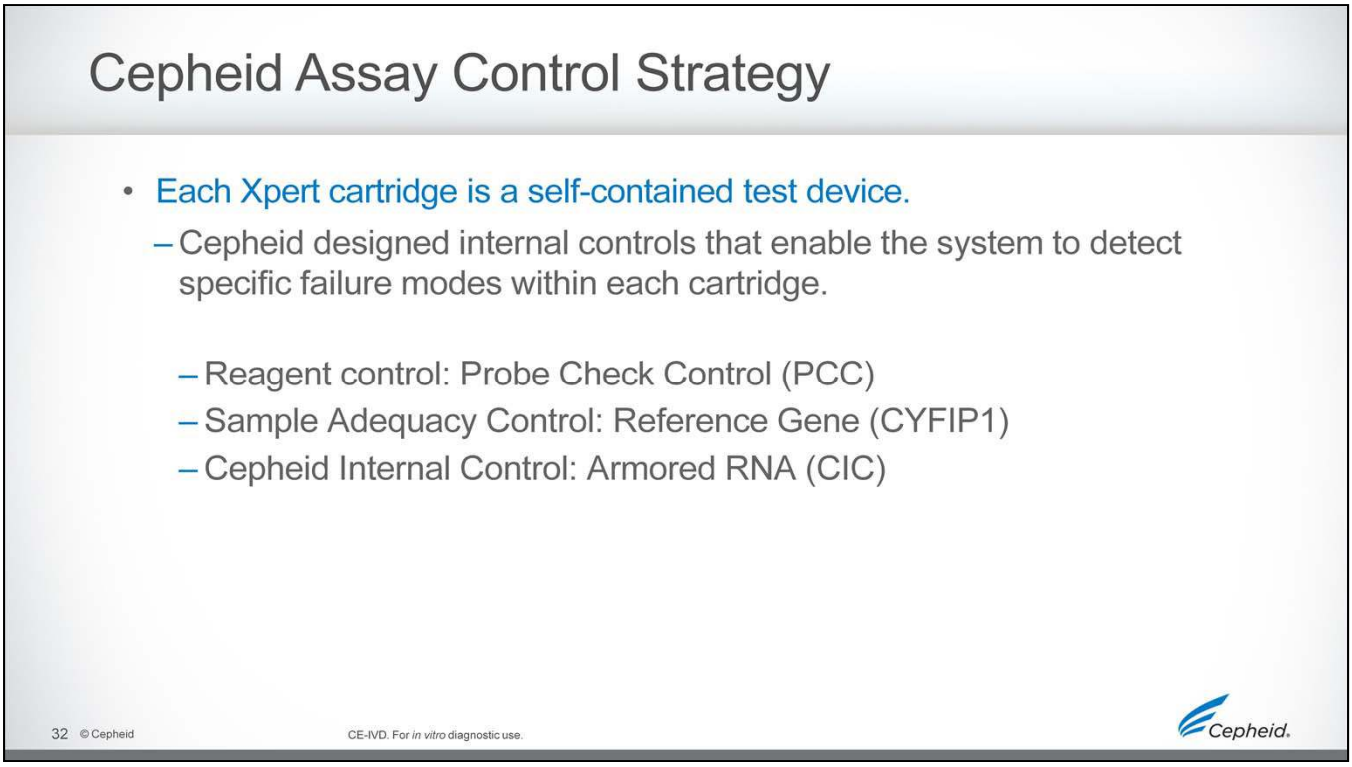
- The Instrument System control checks the optics, temperature of the module, and mechanical integrity of each cartridge.
  - If the system controls fail, an ERROR test result will be reported.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.



- **Assay Name Quality Controls**
  - Each Xpert cartridge is a self-contained test device
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge
    - Specimen Processing Control (SPC)
    - Probe Check Controls (PCC)

To FAS: please refer to document 301-4868 GeneXpert Quality Control features for cepheid Xpert Assays

Probe Check Control – (PCC)

- After sample preparation, bead reconstitution, and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures.
- The readings are compared to default settings established by Cepheid.
- The Probe Check controls for:
  - Missing Target Specific Reagent (TSR) and/or Enzyme Reagent beads, which contain all primers, probes, and internal control templates
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Probe degradation
- If the Probe Check fails, an ERROR test result will be reported.

 © Cepheid CE-IVD. For *in vitro* diagnostic use. Cepheid.

Probe Check Controls (PCC)

- Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor

- bead rehydration
- reaction tube filling
- probe integrity
- dye stability

Sample Adequacy Control – SAC

- Sample Adequacy Control (SAC) verifies that human cells and human RNA have been added into the sample chamber. The SAC passes if it meets the validated acceptance criteria.
- CYFIP1 Control: Cytoplasmic FMR1 interacting protein 1
  - 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 negative SAC may be due to:
  - Insufficient RNA -Improper sample collection
  - Inefficient sample lysis -Insufficient mixing of the sample
- If the SAC fails in an analyte negative sample, an INVALID test result will be reported.

Cepheid Internal Control– CIC

- The Cepheid Internal Control (CIC) ensures that the sample was processed correctly.
- CIC is Armored RNA®.
- Verifies that the RT-PCR reaction proceeded with minimal inhibition.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

STRAT4 External Controls*

Vendor	Control Name	Description	configuration	Storage Temp
Horizon DX** www.horizondiscovery.com	**STRAT4 FFPE Control A**	ESR1 POS PGR POS ERBB2 NEG MKi67 POS	Sectioned slides	4°C
	STRAT4 FFPE Control B	ESR1 POS/NEG PGR POS ERBB2 POS MKi67 POS/NEG	Sectioned slides	4°C
	STRAT4 FFPE Control C	ESR1 NEG PGR NEG ERBB2 NEG MKi67 POS/NEG	Sectioned slides	4°C

*External Controls are still being developed.
** **Alternative vendor: ATCC**
Please note: for negative samples, a lack of human cells will result in an INVALID

External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable

 © Cepheid CE-IVD. For *in vitro* diagnostic use.

Cepheid.

- Though we have already included controls in each cartridges to ensure quality assured results, still if you wish to run your own external controls – you may purchase them from any external provider as per details on screen.
- ***No need to read full slide – just give them an idea and let them self explore if they need it.***

Cepheid.
A better way.

FFPE STRAT 4 External Control Configuration

	The Xpert Breast Cancer STRAT4 FFPE Control		
Targets	**STRAT4 FFPE Control A**	**STRAT4 FFPE Control B**	**STRAT4 FFPE Control C**
ESR1	pos	pos (close to cutoff)	neg
PGR	pos	pos	neg
ERBB2	neg	pos	neg
MKi67	pos	pos (close to cutoff)	pos

Image by Cepheid

STRAT4 FFPE Control A		**STRAT4 FFPE Control B**		**STRAT4 FFPE Control C**	

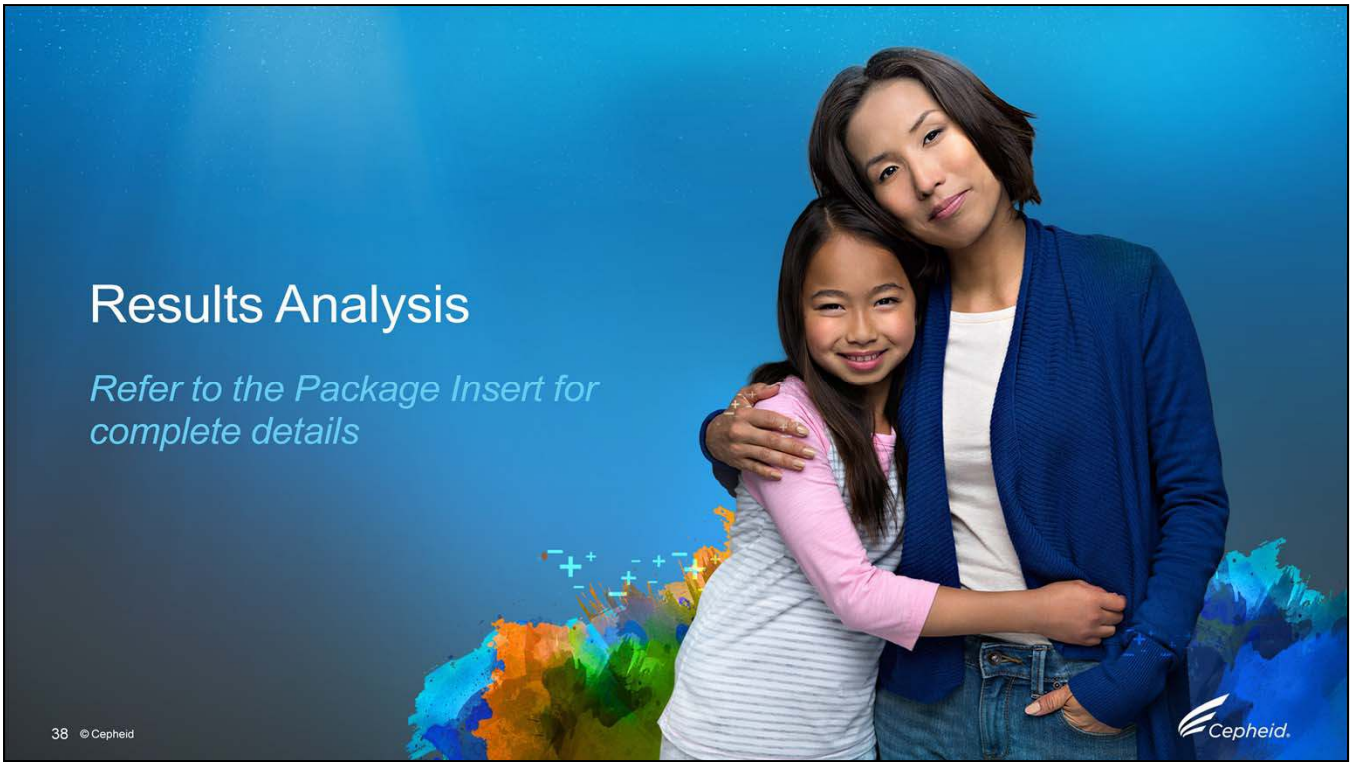
Package:
FFPE Controls, Instructions, Certificate of Analysis

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.



Cepheid.
A better way.

Results

- Xpert Breast Cancer STRAT4 provides POSITIVE or NEGATIVE test result for each target.
- Final report will display each target's individual result.
- A target is considered Positive when it has mRNA overexpression relative to the reference gene.
- For ESR1 and ERBB2 to have a valid result, the CYFIP1 Control must PASS.
- For PGR and MKi67 to have a valid result, the CYFIP1 control must PASS and the CYFIP1 alternate must be POS.
- If the CYFIP1 alternate is NEG, PGR, and MKi67 will be Indeterminate.

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

Results Summary

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

40 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

- Consequently, if you were the software, how would you interpret these results?

Cepheid. A better way.

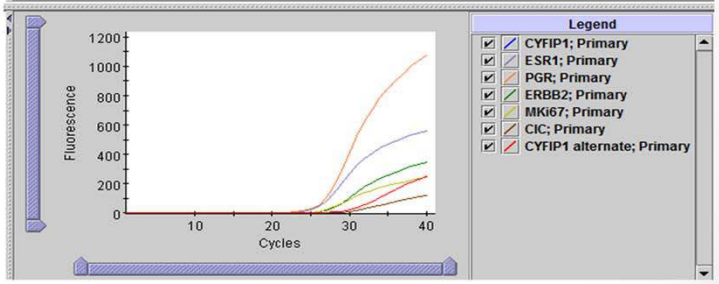
POSITIVE

Test Result ESR1 POSITIVE; PGR POSITIVE; ERBB2 POSITIVE; MKi67 POSITIVE

- The target mRNA transcript is overexpressed and has a delta Ct above the cutoff setting
- CYFIP1 PASS; mRNA transcript has a Ct within the valid range and endpoint above the threshold
- PCC PASS; all probe check results pass

Test Result | Analyte Result | Detail | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	Target Delta Ct
CYFIP1	29.8	247	PASS	PASS	
ESR1	24.8	563	POS	PASS	5.0
PGR	24.5	1079	POS	PASS	5.4
ERBB2	27.1	347	POS	PASS	2.8
MKi67	26.8	244	POS	PASS	3.1
CIC	31.2	119	POS	PASS	
CYFIP1 alternate	29.8	247	POS	PASS	



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

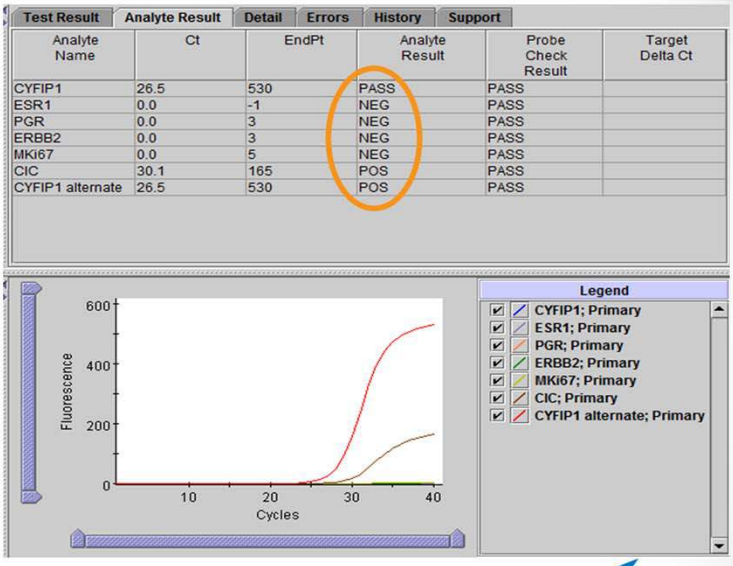
Cepheid

Cepheid
A better way.

NEGATIVE

Test Result	ESR1 NEGATIVE; PGR NEGATIVE; ERBB2 NEGATIVE; MKi67 NEGATIVE

- Target mRNA transcript is not overexpressed and has a delta Ct below the cutoff setting.
- CYFIP PASS; mRNA transcript has a Ct within the valid range and endpoint above the threshold setting.
- PCC PASS; all probe check results pass



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

Mixed Results

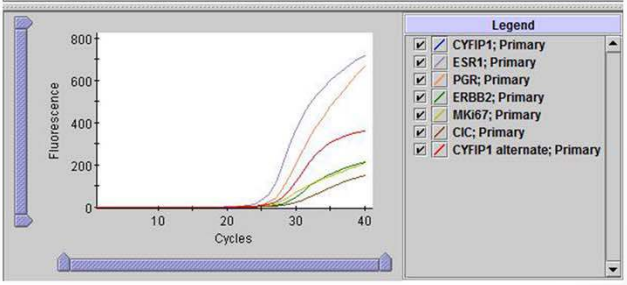
- The target is positive or negative for mRNA overexpression relative to the reference gene.
- CYFIP1 mRNA transcript has a Ct within the valid range and endpoint above the threshold
- PCC PASS; all probe check results pass

Test Result

ESR1 POSITIVE;
PGR POSITIVE;
ERBB2 NEGATIVE;
MKi67 POSITIVE

Test Result | Analyte Result | Detail | Errors | History | Messages | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	Target Delta Ct
CYFIP1	26.8	360	PASS	PASS	
ESR1	24.3	717	POS	PASS	2.5
PGR	25.9	668	POS	PASS	0.8
ERBB2	28.5	214	NEG	PASS	-1.7
MKi67	27.4	206	POS	PASS	-0.7
CIC	29.9	152	POS	PASS	
CYFIP1 alternate	26.8	360	POS	PASS	



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

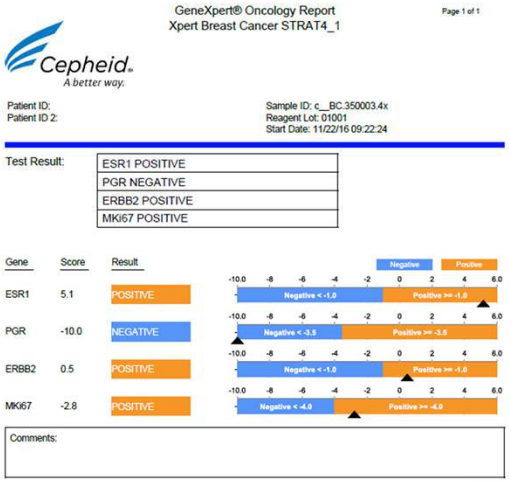
Cepheid.

Cepheid.
A better way.

ONCore Software

- OnCore software is loaded onto Gx Computer

- Turns the GxDx or Xpertise results into a report.
- Provides graphical representation of the data.
- Shows how close the result for each target is to the cutoffs.
- Requires a unique .onc file which is located on the assay ADF disk



GeneXpert® Oncology Report
Xpert Breast Cancer STRAT4_1

Page 1 of 1

Cepheid
A better way.

Patient ID:
Patient ID 2:

Sample ID: c__BC.350003.4x
Reagent Lot: 01001
Start Date: 11/22/16 09:22:24

Test Result:

ESR1 POSITIVE
PGR NEGATIVE
ERBB2 POSITIVE
MKi67 POSITIVE

Gene	Score	Result
ESR1	5.1	POSITIVE
PGR	-10.0	NEGATIVE
ERBB2	0.5	POSITIVE
MKi67	-2.8	POSITIVE

Negative | Positive

-10.0 -8 -6 -4 -2 0 2 4 6.0

ESR1: Negative < -1.0 | Positive >= -1.0
PGR: Negative < -3.5 | Positive >= -3.5
ERBB2: Negative < -1.0 | Positive >= -1.0
MKi67: Negative < -4.0 | Positive >= -4.0

Comments:

 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

Reasons to Repeat the Assay with Concentrated Lysate

- An **INDETERMINATE** result indicates that the sample contained insufficient material for PGR and/or MKi67 result analysis.
- An **INVALID** result indicates that the reference control failed.
  - Sample was not properly processed
  - PCR was inhibited
  - RNA quality in the tumor accessed was inadequate
- In either of these two cases, repeat the test with a more concentrated FFPE lysate.

 © Cepheid CE-IVD. For *in vitro* diagnostic use.

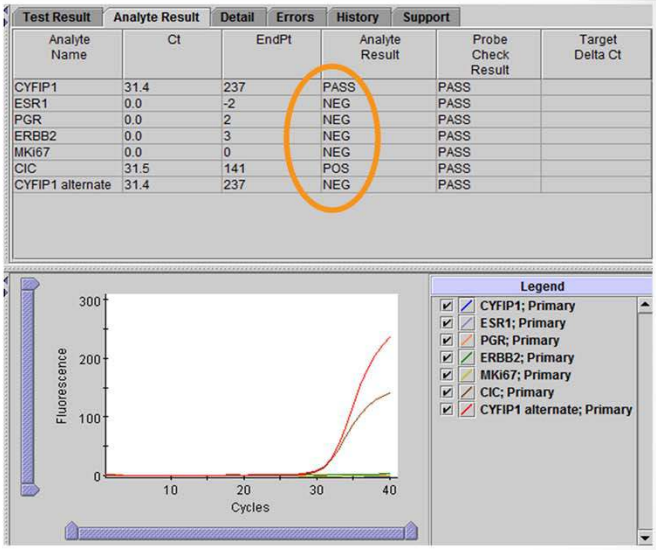
- Reasons to Repeat the Assay
- • An **INVALID** result indicates that the controls SPC failed. The sample was not properly processed or PCR is inhibited.
- • An **ERROR** result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being
- filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.
- • A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

INDETERMINATE

Test Result	ESR1 NEGATIVE; PGR INDETERMINATE; ERBB2 NEGATIVE; MKi67 INDETERMINATE

Expression level of PGR and/or MKi67 mRNAs cannot be determined.

- CYFIP1 alternate – NEG – cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting necessary
- PCC PASS; all probe check results pass



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

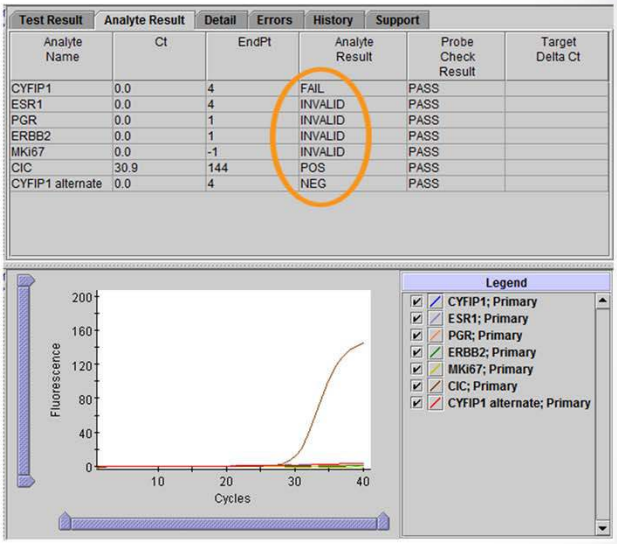
INVALID

Test Result: INVALID

Expression levels of target mRNAs cannot be determined.

- CYFIP1 – FAIL – cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting.
- CYFIP1 alternate - NEG

- PCC PASS; all probe check results pass



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid. A better way.

Xpert Breast Cancer STRAT4 Retest Procedure -for Indeterminate or Invalid Results

Xpert® Breast Cancer STRAT4 **RETEST PROCEDURE**

for Indeterminate or Invalid Results – Concentrated FFPE Lysate Preparation

Cepheid. A better way.

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support
US office
(866) 838-3222, Option 2
techsupport@cepheid.com

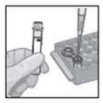
European office
+33 563 82 53 19
support@cepheideurope.com

1 Scrape tissue section or macrodissection from slide.

2 Transfer tissue to provided 1.5 mL tube.

3 Add 260 µL of FFPE lysis reagent to the 1.5 mL tube containing FFPE tissue.

4 Add 5 µL of Proteinase K (PK) to the same 1.5 mL tube. Close lid.



5 Vortex at maximum setting for 5 seconds.

6 Briefly microcentrifuge the tube to remove liquid from the lid.

7 Incubate the sample at 80 °C for 30 minutes.

8 Vortex at maximum setting for 5 seconds.

9 Briefly microcentrifuge the tube to remove liquid from the lid.

10 Add 260 µL of ≥ 95% ethanol to the same 1.5 mL lysis tube.

11 Close lid and vortex the sample at maximum setting for 15 seconds.

12 Briefly microcentrifuge the tube to remove liquid from the lid.

NOTE: Prepared lysate, with ethanol, is stable at 2 to 8 °C, for up to 1 week. And at ≤ -20 °C for up to 4 weeks.

© 2017 Cepheid

301-8035, Rev. A March 2017

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

Reasons to Repeat the Assay with Retained Lysate

- A **REPEAT TEST** result indicates that the internal control (CIC) failed. The sample was not properly processed.
- An **ERROR** result indicates that the Probe Check control failed, possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, maximum pressure limits were exceeded, or a valve positioning error was detected.
- 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 these cases, repeat the test using a new 520µL aliquot of the retained FFPE lysate.

 © Cepheid CE-IVD. For *in vitro* diagnostic use. Cepheid.

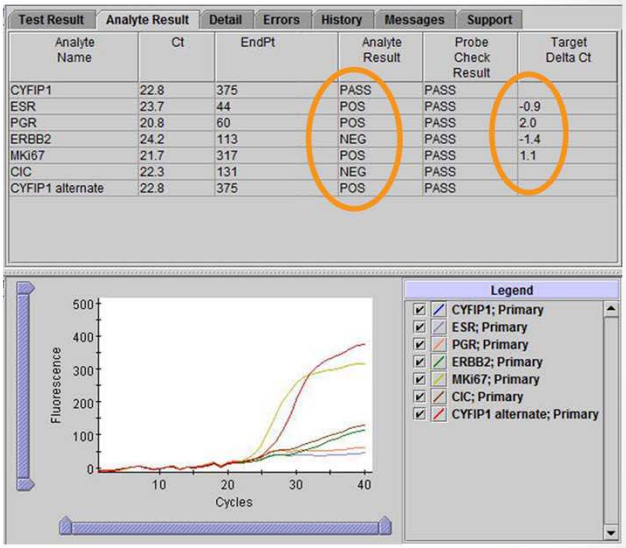
- Reasons to Repeat the Assay
- • An **INVALID** result indicates that the controls SPC failed. The sample was not properly processed or PCR is inhibited.
- • An **ERROR** result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being
- filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.
- • A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Cepheid. A better way.

REPEAT TEST

Test Result | **REPEAT TEST**

- The expression levels of target mRNAs cannot be determined.
- CIC NEG; the internal control has a cycle threshold (Ct) outside the valid range.



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

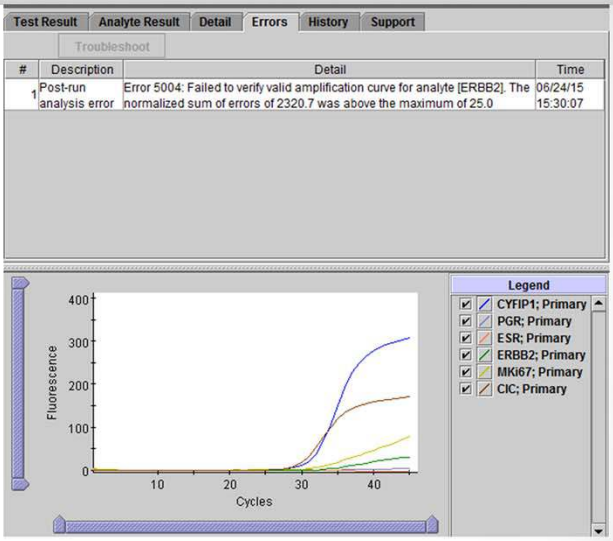
Cepheid.
A better way.

ERROR

Test Result: ERROR

- The expression levels of target mRNAs cannot be determined.
- Probe Check FAIL*; all or one of the probe check results fail.

*If the probe check passed, the error is caused by exceeding the maximum pressure limit, a curve fit error or a system component failure.



 © Cepheid

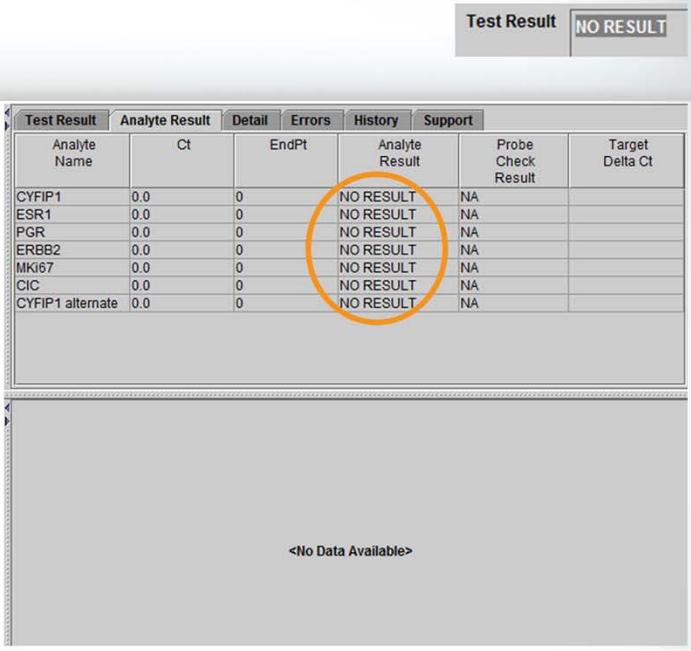
CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

NO RESULT

- Expression levels of target mRNAs cannot be determined.
- A NO RESULT indicates that insufficient data were collected.
  - For example, the operator stopped a test that was in progress.
- PCC NA (not applicable)



 © Cepheid

CE-IVD. For *in vitro* diagnostic use.

Cepheid

Cepheid
A better way.

Xpert Breast Cancer STRAT4 Retest Procedure -for No Result, Repeat Test, or Error Results

Xpert® Breast Cancer STRAT4 **RETEST PROCEDURE**

for No Result, Repeat Test or Error Results

Cepheid. A better way.

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

Cepheid Technical Support
US office
(888) 838-3222, Option 2
techsupport@cepheid.com

European office
+33 563 82 53 19
support@cepheideurope.com

1 Obtain one new cartridge and the retained FFPE lysate for each sample to be re-tested.

2 Vortex FFPE lysate for 15 seconds.

3 Open the Xpert cartridge lid.

4 Pipet 520 µl of retained lysate from the 5 mL vial and transfer into the sample chamber.

5 Close the Xpert cartridge lid.

6 Start the assay within the timeframe specified in the package insert.

© 2017 Cepheid

301-8035, Rev. A March 2017

CE-IVD. For *in vitro* diagnostic use.

Cepheid.

Cepheid. A better way.

- **Read out the slide – not much elaboration required.**

Limitations

- Refer to the Package Insert for a complete list of limitations.

CE-IVD. For *in vitro* diagnostic use.

Technical Support

- Cepheid provides technical support in the field, on the phone, by fax, and by email.
- Contact information for Cepheid offices is available at **http://www.cepheid.com/support**
  - Select the Contact Us option to access contact information
  - Complete online form to Create a Support Case
- Before contacting Cepheid Technical Support, collect the following information:
  - Product name
  - Lot number
  - Serial number of the instrument
  - Error messages (if any)
  - Software version and, if applicable, Computer Service Tag Number

 © Cepheid CE-IVD. For *in vitro* diagnostic use.

- Please prepare the following information before contacting Cepheid Technical Support.
- The global Technical support addresses can be found in the list.
- To FAS: Guide the customer and show how to log a case: click on the link and select language.

Cepheid
A better way.