Assay Training: Xpert® Bladder Cancer Monitor

Technical Training for CE-IVD product only

301-7271, Rev. A August 2016

Training Agenda

- Xpert Bladder Cancer Monitor Training
  - Clinical Utility
  - Reagents
  - Sample collection
  - Kit storage and handling
  - Precautions
  - Preparing cartridge
- Quality Control
- Results analysis

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Training Objectives

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

- Properly store and handle the Xpert Bladder Cancer Monitor cartridge kit.
- Follow proper laboratory safety precautions.
- Collect appropriate specimen types and transport specimen.
- Perform the cartridge set up and run the assay.
- Report the various software-generated results.
- Understand the assay control strategy.

Bladder Cancer

Bladder Cancer Facts:

- Urothelial bladder cancer (UBC) is the 7th most prevalent cancer among men and the 17th most prevalent cancer in women worldwide. UBC is more prevalent in developed countries and is the 4th and 9th most prevalent cancer in men and women, respectively, in the Western world.
- Seventy-five percent of newly diagnosed UBCs are non-muscle invasive cancers while 25% of the remaining diagnoses are muscle invasive, requiring radical interventions.
- The frequency of UBCs combined with the highest recurrence rate of all cancers adds a tremendous cost burden to healthcare systems. The prevalence rate of UBC is the highest of all urological cancers.

References

1. Burger M et al, Epidemiology and Risk Factors of Urothelial Bladder Cancer. Eur Urol 63 (2013) 234-241.

What is Bladder Cancer?

- Bladder cancer mostly affects people over 60 years of age in the United States and Europe. The incidence rates are nearly four times higher in men than in women and are highest in the Caucasian race.[2]
- Bladder cancer has the highest recurrence rate of any malignancy, often as high as 70% within 5 years of successful treatment. While the majority of patients with bladder cancer can be successfully treated with organ-sparing therapy, most will experience either a recurrence or progression. [3]

References

2. Siegel R, Naishadham D, Jemal A. Cancer Statistics, 2012. CA: Cancer J Clin, 2012, 62(1): 10-29

3. Hollenbeck BK, Dunn RL, Ye Z, Hollingsworth JM, Skolarus TA, Kim SP, Montie JE, Lee CT, Wood DP Jr, Miller DC. Delays in diagnosis and bladder cancer mortality. Cancer 2010, 116(22):5235-42.

The Need:

- Bladder cancer monitoring test that provides:
  - an aid to standard clinical evaluation in monitoring for bladder cancer recurrence in patients previously diagnosed with bladder cancer and should be used in conjunction with other clinical measures to assess disease recurrence
  - Improved sensitivity
  - Ability to confidently rule out bladder cancer
  - Platform that is easy to use and learn
  - Noninvasive specimen type

Urologists are managing increasing workloads with the need to drive timely treatment decisions.

The Cepheid Solution

- 5 mRNA targets detected (ABL1, ANXA10, UPK1B, CRH, and IGF2). ABL1 functions as SAC
- 93.9% NPV **compared to cystoscopy and histology**
- Simple and Easy to Use
- 3 internal controls for each sample
  - Probe Check control (PCC)
  - Cepheid Internal Control (CIC)
  - Sample Adequacy Control (SAC)

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Xpert® Bladder Cancer

Intended Use

Xpert® Bladder Cancer Monitor, is a qualitative *in vitro* diagnostic test intended to monitor for the recurrence of bladder cancer in patients previously diagnosed with bladder cancer. The test utilizes a voided urine specimen and measures the level of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) by means of real-time, reverse transcription-polymerase chain reaction (RT-PCR).

Xpert Bladder Cancer Monitor is indicated as an aid to standard clinical evaluation in monitoring for bladder cancer recurrence in patients previously diagnosed with bladder cancer and should be used in conjunction with other clinical measures to assess disease recurrence.

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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)

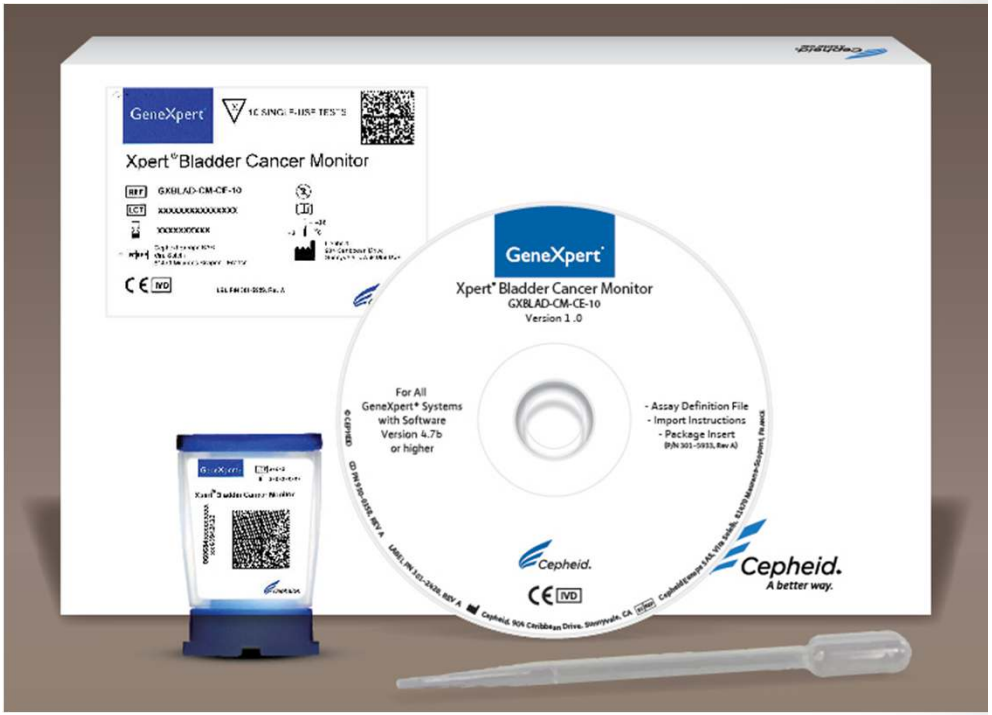
- GXBLAD-CM-CE-10

Materials Required but not Provided

- Xpert Urine Transport Reagent Kit catalog# GXUTR-CE-30
- Urine collection cup

Xpert Bladder Cancer Kit Components

	Xpert Bladder Cancer
Catalog Number	**GXBLAD-CM-CE-10**
Tests per kit	10
Contents per test cartridge	Reagent beads
	Elution Reagent
Kit CD	Assay Definition File (ADF) -.gxa
	Instruction to import ADF
	Package insert (multi-languages)
Transfer pipettes	10
Storage	2-28° C



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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.

Good Laboratory Practice, cont'd

Housekeeping	• Clean work surfaces with a final concentration of 1:10 dilution of household bleach* in water and then a 70% ethanol solution. Wipe work surfaces dry. • If contamination occurs, thoroughly clean the contaminated area with 1:10 dilution of household bleach* in water or 3% (w/v) hydrogen peroxide and rinse thoroughly with water. Wipe work surfaces dry.
Personnel	• Wear clean lab coats and gloves. • Change gloves between processing samples.
Lab bench area	• Clean the lab bench area routinely. • Keep the back of the instrument dust free.

** Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*

Xpert Bladder Cancer Kit Storage and Handling

- Store test kits at 2-28ºC. Do not use expired cartridges.
- Each single-use cartridge is used to process one test. Do not reuse processed cartridges.
- Do not open a cartridge until ready to use.
  - Start the test within 30 minutes of adding the sample to the cartridge. Infinity on-board stability is 4 hours.
- Avoid cross contamination during sample handling steps.
  - Change gloves between samples.
- Do not use a cartridge that has been dropped or shaken. Shaking or dropping the cartridge after opening the lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use a cartridge that has leaked.
- Do not substitute other reagents with the Xpert Bladder Monitor Assay.
- Do not use any cartridge that has contents that have become cloudy or discolored.

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Warnings and Precautions:

- Treat all biological samples, including used cartridges, with standard precautions.
- Urine samples must be treated with Xpert Urine Transport Reagent.
  - Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and reagents.
  - Wash hands thoroughly after handling specimens and test reagents.
- Urine Transport Reagent contains guanidinium chloride which is

  toxic if swallowed (H302).
- Follow your institution's safety procedures for working with chemicals and handling biological samples.

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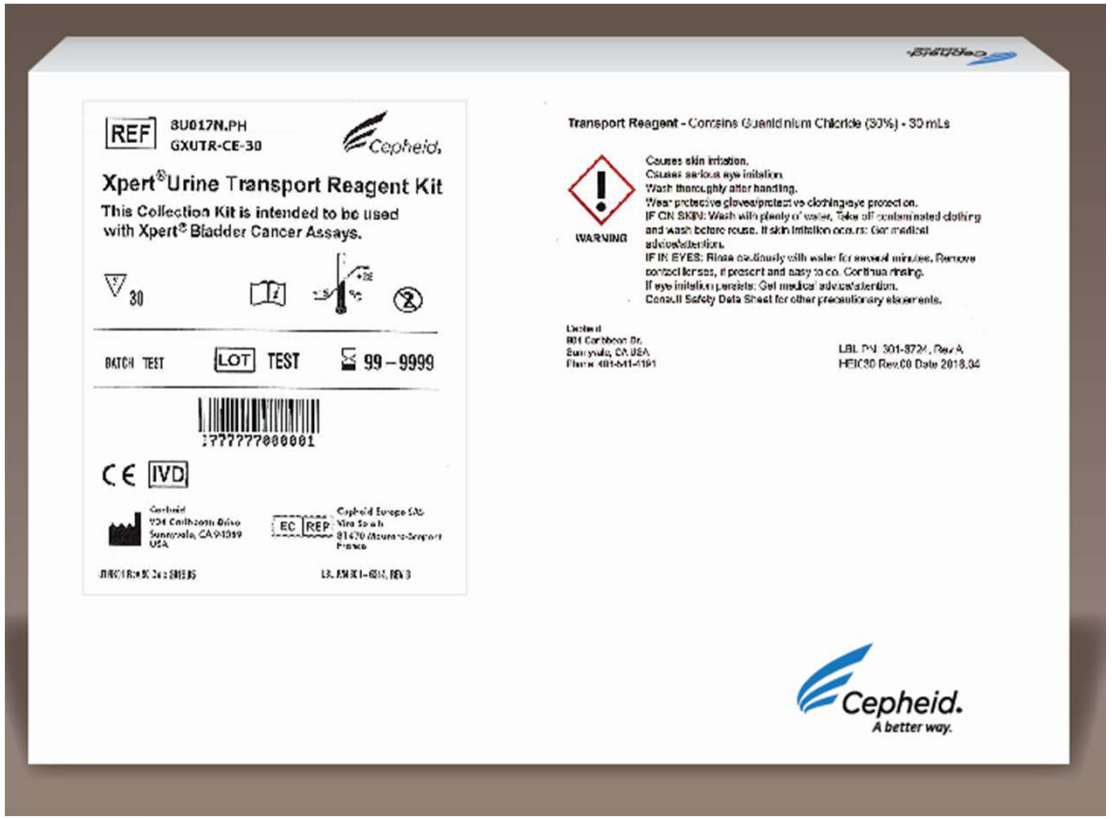
Specimen Collection

Specimen Collection Kit



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Xpert Bladder Transport Reagent Kit- Catalog # GXUTR-CE-30

Kit Contents:

- Tube containing 4.5mL of preservative
- Transfer pipette

4.5mL of voided urine is added to the tube

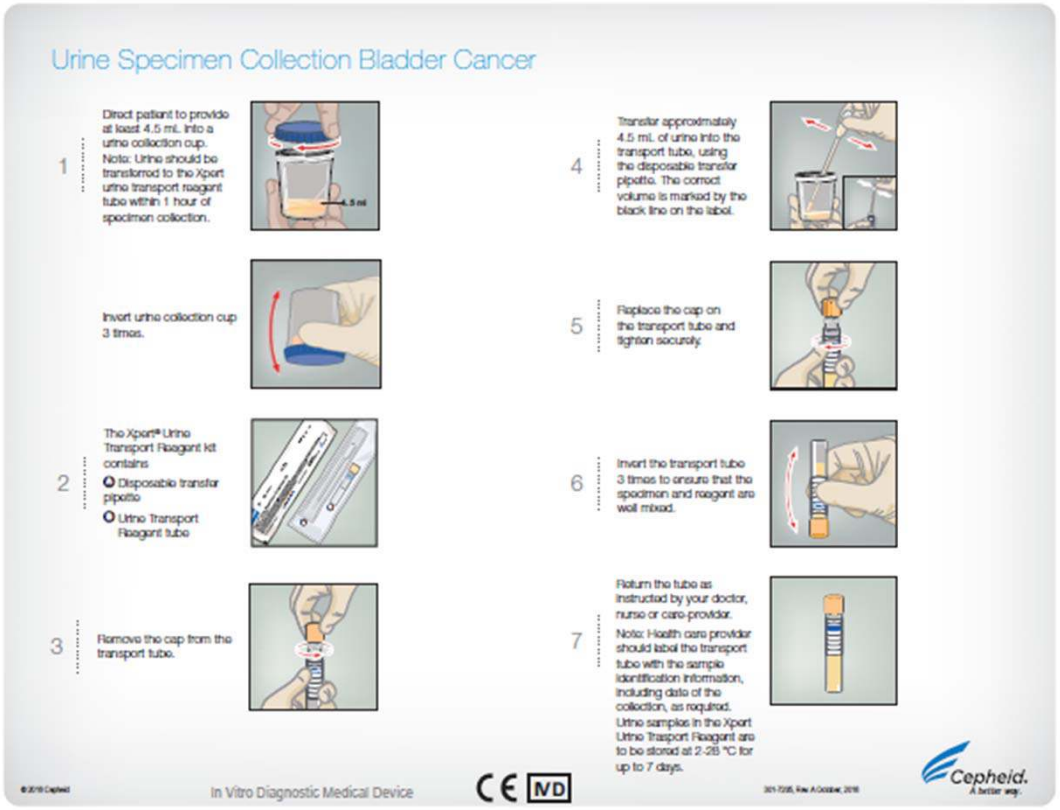
The preservative + urine volume is sufficient for one test and one repeat test if needed.

Xpert Bladder Cancer Specimen Transport and Storage

- **Urine samples should be transferred to Xpert Urine Transport Reagent tubes within 1 hour of primary collection.**

Specimen	Transport and Storage Temperature (˚C)	Storage Time
Urine in Xpert Urine Transport Reagent	2-28 ˚C	7 days

Sample Collection Card



Urine Specimen Collection Bladder Cancer

1 Direct patient to provide at least 4.5 mL into a urine collection cup.
Note: Urine should be transferred to the Xpert urine transport reagent tube within 1 hour of specimen collection.

Invert urine collection cup 3 times.

2 The Xpert® Urine Transport Reagent kit contains
- Disposable transfer pipette
- Urine Transport Reagent tube

3 Remove the cap from the transport tube.

4 Transfer approximately 4.5 mL of urine into the transport tube, using the disposable transfer pipette. The correct volume is marked by the black line on the label.

5 Replace the cap on the transport tube and tighten securely.

6 Invert the transport tube 3 times to ensure that the specimen and reagent are well mixed.

7 Return the tube as instructed by your doctor, nurse or care-provider.
Note: Health care provider should label the transport tube with the sample identification information, including date of the collection, as required.
Urine samples in the Xpert Urine Trasport Reagent are to be stored at 2-28 °C for up to 7 days.

In Vitro Diagnostic Medical Device

CE IVD

301-7205, Rev. A October, 2010

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Xpert Bladder Cancer Cartridge Preparation

Xpert® Bladder Cancer Cartridge Preparation depicting urine sample in primary sample cup

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

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1 Obtain a urine sample of at least 4.5mL in the primary collection cup.

2 Obtain one Xpert Urine Transport Reagent kit.

3 Invert cup 3 times to mix the urine.

4 Use the disposable transfer pipette provided in the Xpert Urine Transport Reagent kit in the pouch with the tube. NOTE: Do not use the pipette from the assay kit.

5 Insert the transfer pipette into the bottom of the urine cup.

6 Transfer approximately 4.5 mL of urine so that the volume reaches the black line on the tube label.

7 Replace the cap on the Xpet Urine Transport Reagent tube and tighten securely. Label correctly and transfer to the GeneXpert cartridge preparation area.

8 See reverse side of this card for further instructions.

301-7364, Rev. A October, 2016

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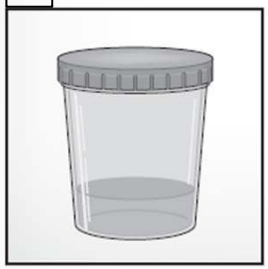
Bladder Cancer Cartridge Preparation

1

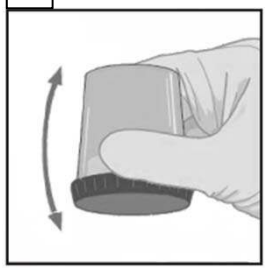


Obtain a urine sample of at least 4.5 mL in the primary collection cup.

2

Obtain one Xpert Urine Transport Reagent Kit.

3



Invert cup 3 times to mix the urine.

4

Note: DO NOT use the pipette from the assay kit.

Use the disposable transfer pipette provided in the Xpert Urine Transport Reagent kit.

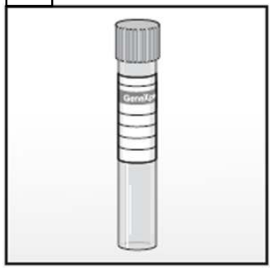
5

Insert the transfer pipette into the bottom of the urine cup.

6

Transfer approximately 4.5 mL of urine so that the volume reaches the black line on the tube label.

7



Replace the cap on the Urine Transport Reagent tube and tighten securely. Invert 3 times to mix.

8

See reverse side of this card for further instructions.

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Xpert Bladder Cancer Cartridge Preparation (cont.)

Xpert® Bladder Cancer Cartridge Preparation depicting urine sample

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

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Cepheid. A better way.

1 Obtain one appropriately collected and labeled urine sample.

2 Obtain one Xpert Bladder Cancer cartridge and transfer pipette (provided).

3 Open the Xpert cartridge lid.

4 Gently invert the transport tube 3 times to mix.

5 Fill the transfer pipette with sample up to the 4 mL mark.

6 Empty the pipette contents into the sample chamber.

7 Close the Xpert cartridge lid.

8 Insert the cartridge and start the assay.

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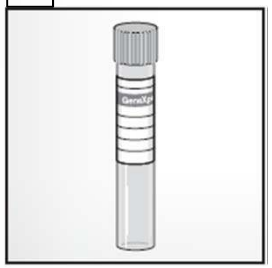
Bladder Cancer Cartridge Preparation

1

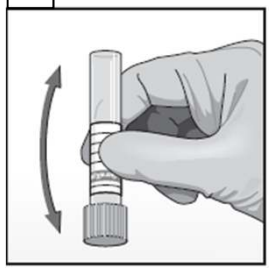


Obtain one appropriately collected and labeled urine sample.

2

Obtain one Xpert Bladder Cancer cartridge and transfer pipette (provided).

3

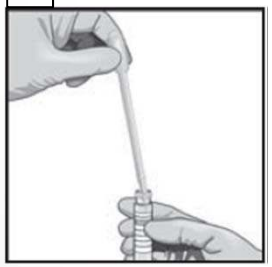


Gently invert the transport tube 3 times to mix.

4

Open the Xpert cartridge lid.

5



Fill the transfer pipette with sample up to the 4 mL mark.

6

Empty the pipette contents into the sample chamber.

7

Close the Xpert cartridge lid.

8

Place the cartridge on the instrument and start the assay.

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Assay Procedure – Cartridge Preparation

If using a GeneXpert Dx instrument

- **Start the test within 30 minutes of adding the Sample Reagent-treated 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 Sample Reagent-treated sample to the cartridge.**
- **Remaining on-board stability is tracked by the GeneXpertise® Software so that tests are run prior to the four hour on-board expiration.**

Automated Xpert Bladder Cancer Test Steps

1. Add the sample to the cartridge
2. Place the cartridge into the instrument.
3. Cells are captured and lysed.
4. Cell lysate is mixed with RT-PCR reagents.
5. Simultaneous amplification and detection occurs.
6. Results are ready to view.

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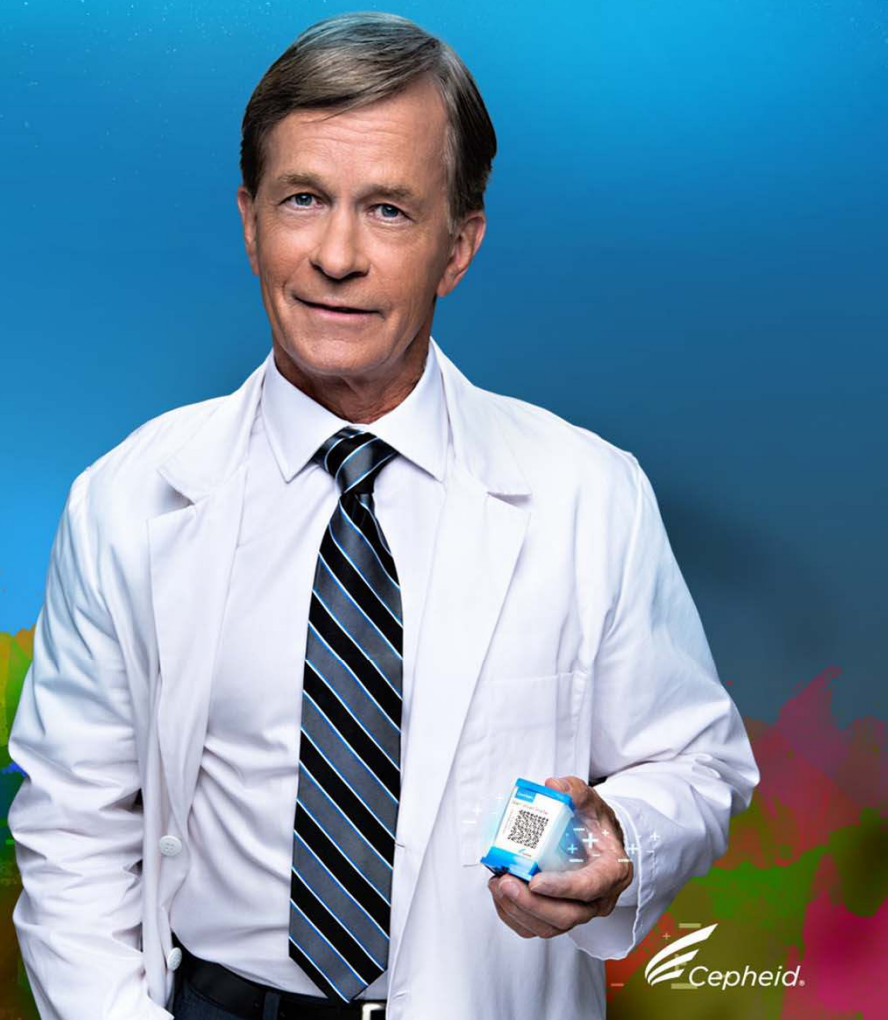
Quality Control

Refer to the Package Insert for complete details



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 Assay Control Strategy

- **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.
    - Reagent control: Probe Check Control (PCC)
    - Sample Adequacy Control: SAC in form of ABL1
    - Cepheid Internal Control: CIC

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.**

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.**
- **ABL1 signal is required for a valid test result.**
- **A negative SAC may be due to:**
  - Insufficient number of human cells
  - Insufficient mixing of the sample
  - Improper sample collection
  - Inefficient sample lysis
- **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 correctly processed.**
- **The CIC passes if it meets the validated acceptance criteria.**
- **CIC is Armored RNA®.**
- **The CIC controls for specimen-associated inhibition.**
- **The CIC can be negative or positive in an analyte-positive sample.**
- **If the CIC fails in an analyte-negative sample, an INVALID test result will be reported.**

Commercially Available External Controls

Vendor	Organism Name	Description	Configuration	Storage Temp
MMQCI Xpert Bladder Cancer QC Panel C104	ABL1 and 4-marker (ANXA10, UPK1B, CRH, and IGF2) *in vitro* transcripts	• Low Positive Control	6x 4.0mL bottles	-20˚C
	ABL1 *in vitro* transcripts	• Negative Control	6x 4.0mL bottles	-20˚C

- **Other options:**
  - **Known positive and negative patient samples**

Please note: for negative samples, a lack of human cells will result in an INVALID

Results Analysis

Refer to the Package Insert for complete details

Linear Discriminant Analysis (LDA)

- **Xpert Bladder Cancer Monitor provides POSITIVE or NEGATIVE test results based on the results of a linear discriminant analysis (LDA) algorithm that utilizes the cycle threshold (Ct) results of the five mRNA targets.**

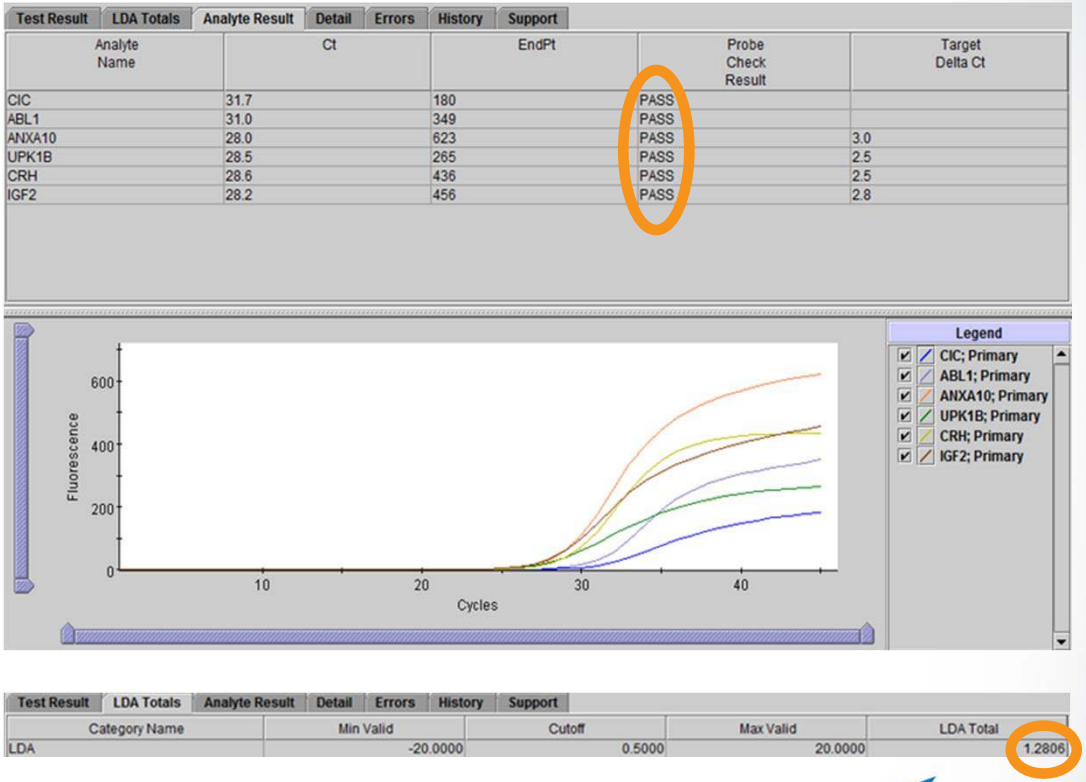
- **It is not necessary to detect all of the mRNA targets for a POSITIVE test result.**

POSITIVE

Test Result POSITIVE

The Linear Discriminant Analysis (LDA) Total (the result of an algorithm that uses the Ct values of ABL1, ANXA10, UPK1B, CRH and IGF2) is equal to, or above the cut off.

- The LDA Total must be within the valid range of -20 to 20.
- ABL1 Ct is within the valid range.
- CIC Not applicable. The CIC results are ignored because the assay targets in positive samples can interfere with this control.
- PCC PASS; all probe check results pass



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NEGATIVE

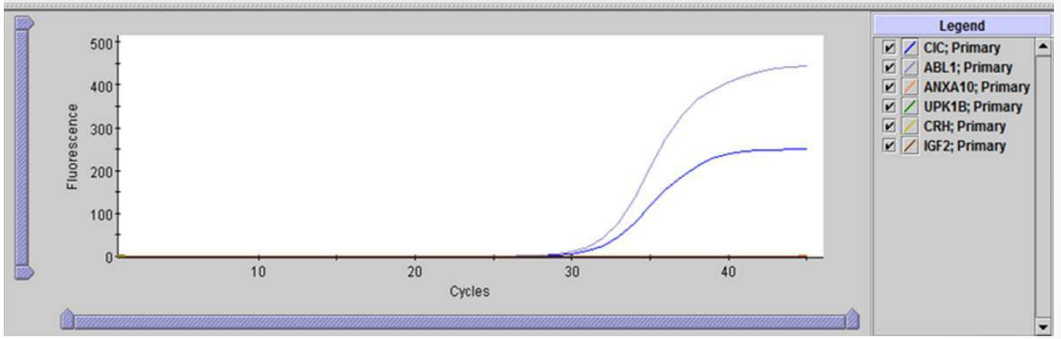
Test Result NEGATIVE

The LDA Total is below the cut off.

- ABL1 Ct is within the valid range. (SAC)
- CIC Ct is within the valid range.
- PCC PASS; all probe check results pass

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

Analyte Name	Ct	EndPt	Probe Check Result	Target Delta Ct
CIC	31.6	250	PASS	
ABL1	31.4	444	PASS	
ANXA10	0.0	1	PASS	-6.6
UPK1B	0.0	-1	PASS	-9.6
CRH	0.0	0	PASS	-7.6
IGF2	0.0	-1	PASS	-8.6



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

Category Name	Min Valid	Cutoff	Max Valid	LDA Total
LDA	-20.0000	0.5000	20.0000	-0.0641

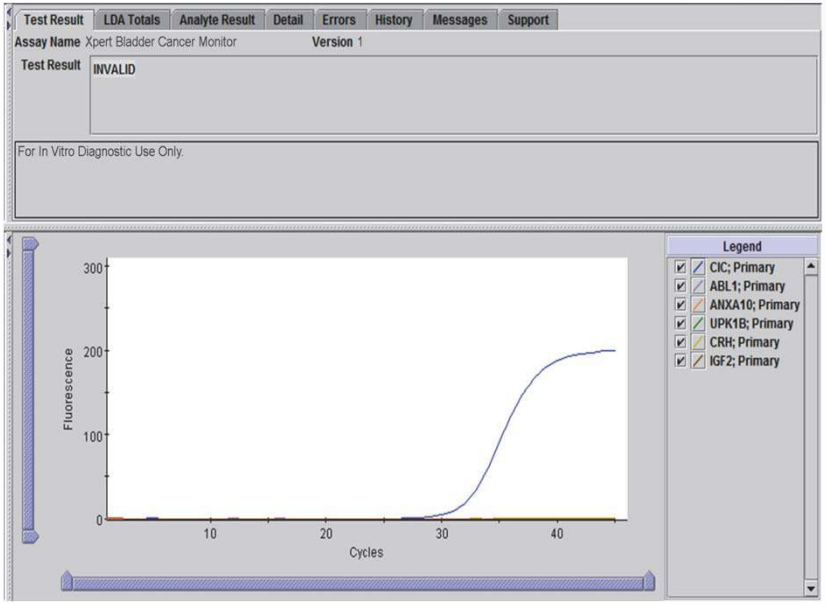
Reasons to Repeat the Assay

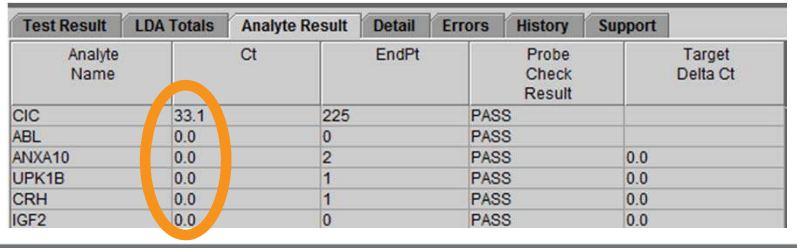
- An **INVALID** result indicates that the sample was not properly processed, PCR was inhibited, or the sample was inadequate.
- An **ERROR** result indicates that the Probe Check Control failed or maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, a load error occurred, or the software was closed prematurely.

INVALID

Presence or absence of target mRNAs cannot be determined.

- ABL1 Ct and /or CIC Ct do not meet acceptance criteria or one or more of the growth curves do not meet acceptance criteria.
- PCC PASS; all probe check results pass



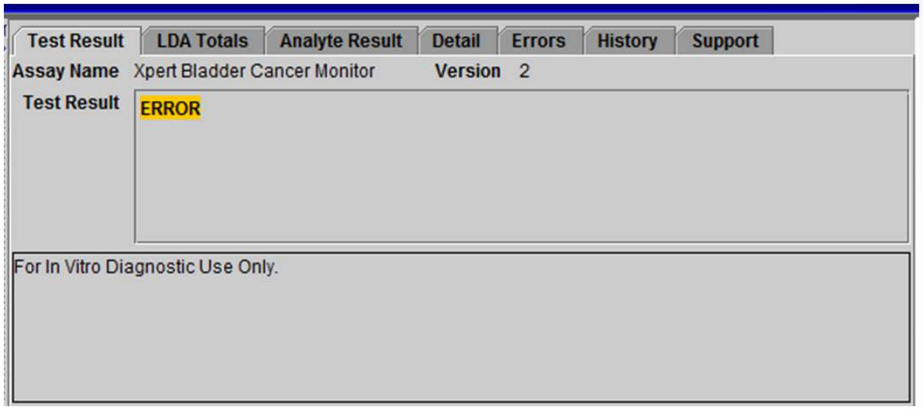


Analyte Name	Ct	EndPt	Probe Check Result	Target Delta Ct
CIC	33.1	225	PASS	
ABL	0.0	0	PASS	
ANXA10	0.0	2	PASS	0.0
UPK1B	0.0	1	PASS	0.0
CRH	0.0	1	PASS	0.0
IGF2	0.0	0	PASS	0.0

ERROR

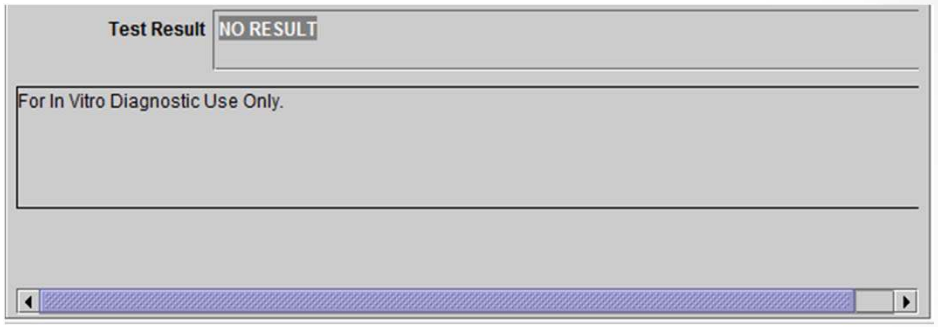
- The presence or absence of target mRNAs cannot be determined.
- Probe Check FAIL*; all or one of the probe check results fail.

*If the probe check passed, the error is caused by a system component failure.



NO RESULT

- Presence or absence 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)



Bladder Cancer Retest Procedure

1	**Discard Used Cartridge.**	
2	• **Obtain the remaining sample in the urine transport reagent.** • **If the leftover sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample.**	
3	**Repeat the test with a new cartridge.**	
4	**Follow the Package Insert on how to run a test.**	

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Factors That Negatively Affect Results

- **Improper specimen collection**
  - Performance with other collection devices and specimen types has not been assessed.
  - For assays that contain the SAC control, a specimen that does not contain human cells will result in an invalid test result if the analyte is negative.
- **Improper transport or storage of collected specimen**
  - Storage and transport conditions are specimen specific.
  - Refer to the Package Insert for the appropriate handling instructions.
- **Improper testing procedure**
  - Modification of the testing procedures may alter the performance of the test.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the Package Insert is necessary to avoid erroneous results.
- **Interfering substance**
  - False negative test results or invalid results may be observed in the presence of interfering substances.
- **The number of human cells in the specimen is below the detection limit of the test**

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Limitations

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

Technical Support

Cepheid provides technical support in the field, on the phone, by fax, and by email.

- **Contact information for Cepheid offices is available on our website at**

http://www.cepheid.com/support

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