GeneXpert®
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Xpert® Bladder Cancer Detection

REF **GXBLAD-CD-CE-10**

Instructions for Use

IVD CE

Cepheid®

In Vitro Diagnostic Medical Device

301-2414, Rev. F
March 2023

Trademark, Patents and Copyright Statements

See Revision History for a detailed list of changes.

Xpert® Bladder Cancer Detection

For *In Vitro* Diagnostic Use Only.

1 Proprietary Name

Xpert® Bladder Cancer Detection

2 Common or Usual Name

Xpert Bladder Cancer Detection

3 Intended Use

Xpert® Bladder Cancer Detection, performed on the Cepheid GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test intended to detect the presence of bladder cancer in adult patients with hematuria suspected of having 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 Detection is indicated as an aid to standard clinical evaluation in the initial diagnosis of bladder cancer in adult patients with hematuria and should be used in conjunction with other clinical measures to assess disease diagnosis.

4 Summary and Explanation

Urothelial bladder cancer (UBC) is the 7th most prevalent cancer among men and the 17th most prevalent cancer in women worldwide.[1] 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.[1] The frequency of UBCs combined with the highest recurrence rate of all cancers adds a tremendous cost burden to healthcare systems. The incidence of UBCs has declined in specific countries and is thought to be linked to a decrease in tobacco use and improved industrial hygiene in high risk occupations. However, the global burden is thought to be on the rise primarily in developing countries, especially in China where smoking is prevalent and the population is high. The prevalence rate of UBC is the highest of all urological cancers.[1] In the United States the incidence of bladder cancer continues to increase in the aging population with the most recent estimates of over 74,000 cases in 2015. In addition, over 500,000 patients are living with bladder cancer in the United States.[2] In the European Union (EU) age standardized incidence rate is 27 per 100,000 for men and six per 100,000 for women. Incidence, prevalence and mortality vary between regions and countries.[1] Bladder cancer mostly affects people over 60 years of age in the United States and Europe.[2] The incidence rates are nearly four times higher in men than in women and are highest in the Caucasian race.[2]

Bladder cancer most commonly presents with microscopic or painless gross hematuria, which is evident in approximately 80-90% of patients diagnosed with bladder cancer. People initially seen with advanced disease are more likely to experience symptoms, such as back or pelvic pain, in addition to hematuria. The clinical presentation of most patients is unremarkable and diagnosis requires more invasive tests.

Bladder cancer has the highest recurrence rate of any malignancy, often as high as 70% within five 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. This high recurrence/progression rate requires diligent and accurate monitoring as a means for early diagnosis and treatment; such monitoring dramatically improves survival.[3]

Currently, there is a need for improved sensitivity and improved turnaround time testing solutions for symptomatic patients. Xpert Bladder Cancer Detection utilizes the Cepheid GeneXpert Instrument Systems to measure the expression of five mRNA targets in a voided urine sample in a self-contained cartridge. This easy to use and fast solution requires less than two minutes of hands-on time with a total turnaround time of approximately 90 minutes.

5 Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time polymerase chain reaction (PCR) and reverse transcriptase assays (RT-PCR). The systems consist of an instrument, personal computer, and preloaded software for performing tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained and samples never come into contact with working parts of the instrument modules, cross-contamination between samples is minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

Xpert Bladder Cancer Detection includes reagents for the detection of five mRNA targets (ABL1, ANXA10, UPK1B, CRH, and IGF2). ABL1 serves as a Sample Adequacy Control (SAC). The ABL1 ensures that the sample contains human cells and human RNA. A positive ABL1 signal is required for a valid test result. A Probe Check Control (PCC) is included to verify reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. A Cepheid Internal Control (CIC), designed to detect sample-associated inhibition of the real-time RT-PCR, is included in each cartridge.

Urine samples must first be treated with the Xpert® Urine Transport Reagent Kit by transferring 4.5 mL of urine to the Urine Transport Reagent tube and inverting three times to mix. The transfer pipette provided with Xpert Bladder Cancer Detection is used to transfer 4 mL of treated urine to the Sample Chamber of the cartridge.

All reagents required for sample preparation and RT-PCR analysis are preloaded in the cartridge. Cells in the urine sample are captured on a filter and lysed by sonication. The released nucleic acid is eluted, 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 90 minutes.

Xpert Bladder Cancer Detection 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.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Bladder Cancer Detection kit contains sufficient reagents to process 10 quality control samples and/or urine samples treated with Xpert Urine Transport Reagent Kit (catalog# GXUTR-CE-30). The Xpert Bladder Cancer Detection kit contains the following:

Xpert Bladder Cancer Detection Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Elution Reagent	1.5 mL per cartridge
Disposable Transfer Pipettes	**1 bag of ten per kit**
CD	**1 per kit**

- Assay Definition File
- Instructions for Use (Package Insert)

Note Safety Data Sheets (SDS) are available at www.cepheid.com or https://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 mixing of the material with other animal materials.

7 Storage and Handling

- Store Xpert Bladder Cancer Detection cartridges and reagents 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 Urine Transport Reagent Kit (catalog# GXUTR-CE-30) for treating primary urine samples. The kit consists of a tube of Xpert Urine Transport Reagent and a transfer pipette.
- GeneXpert Dx Instrument or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, Operator Manual.
  - For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
  - For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher
- 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 specimens should be treated as if they are capable of transmitting infectious agents. All human specimens 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 the Intended Use Section only. The performance of this test with other specimen types or samples has not been evaluated.
- Urine samples must be treated with Xpert Urine Transport Reagent Kit (catalog# GXUTR-CE-30).
- Do not open a Xpert Bladder Cancer Detection cartridge except when adding sample treated with the Xpert Urine Transport Reagent.
- Do not use a cartridge that has been dropped or shaken.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Bladder Cancer Detection cartridge is used to process one test. Do not reuse spent cartridges.
- The single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
- 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

According to Regulation (EC) No. 1272/2008 (CLP), this material is not considered hazardous.

11 Sample Collection, Transport, and Storage

- Use only with urine specimens treated with the Xpert Urine Transport Reagent Kit (catalog# GXUTR-CE-30). Follow the manufacturer's instructions for collecting and handling urine specimens.
- Urine specimens should be transferred to Xpert Urine Transport Reagent tubes within one hour of primary collection. Prior to transferring urine to Xpert Urine Transport Reagent tube, ensure that the urine collection cup has been inverted three times to mix.
- Urine samples stored in Xpert Urine Transport Reagent tubes should be transported to the laboratory at 2–28 °C.
- Urine samples in Xpert Urine Transport Reagent tubes are stable up to seven days at 2–28 °C before testing with Xpert Bladder Cancer Detection.

12 Procedure

12.1 Preparing the Cartridge

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

1. Remove the cartridge from the package.
2. Invert the Xpert Urine Transport Reagent tube three times to mix.
3. Open the cartridge lid.
4. Open the transport tube lid.
5. Fill the sample to the 4 mL mark.
   a) Compress the bulb of the transfer pipette.
   b) Insert the pipette into the transport tube.
   c) Release the bulb to fill the transfer pipette to the 4 mL mark.
   d) Retain the remaining sample at 2–28 °C in case a retest is required.

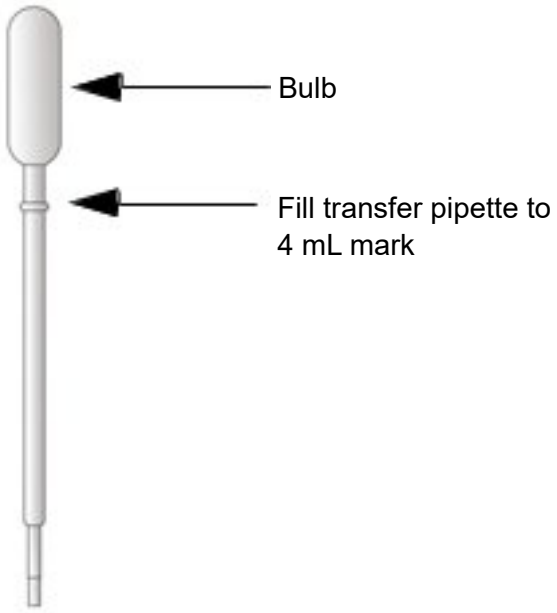


Figure 1. Xpert Bladder Cancer Detection Transfer Pipette

6. Empty the pipette’s content into the Sample Chamber of the cartridge.

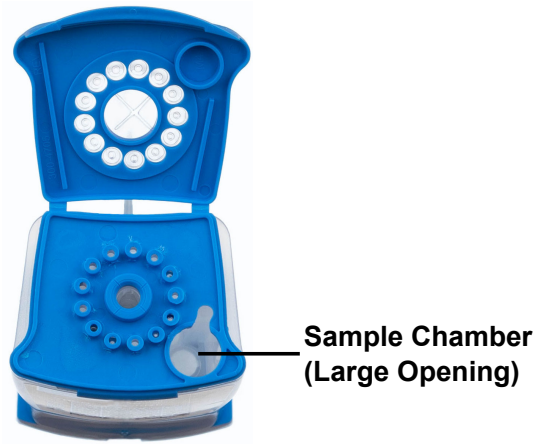


Figure 2. Xpert Bladder Cancer Detection Cartridge (Top View)

7. Close the cartridge lid.

12.2 Starting the Test

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

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

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

1. Turn on the GeneXpert instrument system:
   - If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut 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 shortcut 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 Bladder Cancer Detection 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 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.

   or

   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. Warnings and Precautions.

13 Viewing and Printing Results

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

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

14 Quality Control

Built-in Quality Controls

Each test includes an Internal Control (CIC), Probe Check Control (PCC) and ABL1 Control.

- **Cepheid Internal Control (CIC)**: The CIC is an Armored RNA® control in the form of a dry bead that is included in each cartridge to detect specimen-associated inhibition of the RT-PCR. The CIC passes if it meets the validated acceptance criteria.
- **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.
- **ABL1 Control**: This Sample Adequacy Control (SAC) ensures that the sample contains human cells and human RNA. The ABL1 signal is required for a valid test result. A negative ABL1 indicates that the sample does not contain sufficient human cells or that the sample has degraded.
- **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 by the GeneXpert Instrument System fr om measured fluorescent signals and embedded calculation algorithms and will be shown in the **View Results** window on the Test Results, LDA Totals and Analyte Result tabs. The Test Result, LDA totals and Analyte Results are also shown on the Test Report.

Table 1. Xpert Bladder Cancer Detection Representative Results and Interpretation

Result	Interpretation
POSITIVE See Figure 3 and Figure 4.	• The 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: ABL1 Ct is within the valid range. • CIC: Not applicable. The CIC results are ignored because the test targets in positive samples can interfere with this control. • PCC– PASS; all probe check results pass
NEGATIVE See Figure 5 and Figure 6.	• The LDA Total is below the cut off. • ABL1: ABL1 Ct is within the valid range. • CIC: CIC Ct is within the valid range. • PCC– PASS; all probe check results pass
INVALID See Figure 7 and Figure 8.	Presence or absence of target mRNAs cannot be determined. • ABL1 and CIC: 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 • The cellular content in the sample is too low, PCR was inhibited or the sample was not properly collected.

Result	Interpretation
ERROR	Presence or absence of target mRNAs cannot be determined. • PCC FAIL; all or one of the probe check results fail. • Possible reasons for error include the reaction tube was filled improperly, a reagent probe integrity problem was detected, pressure limits were exceeded, or a valve position error was detected.
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)

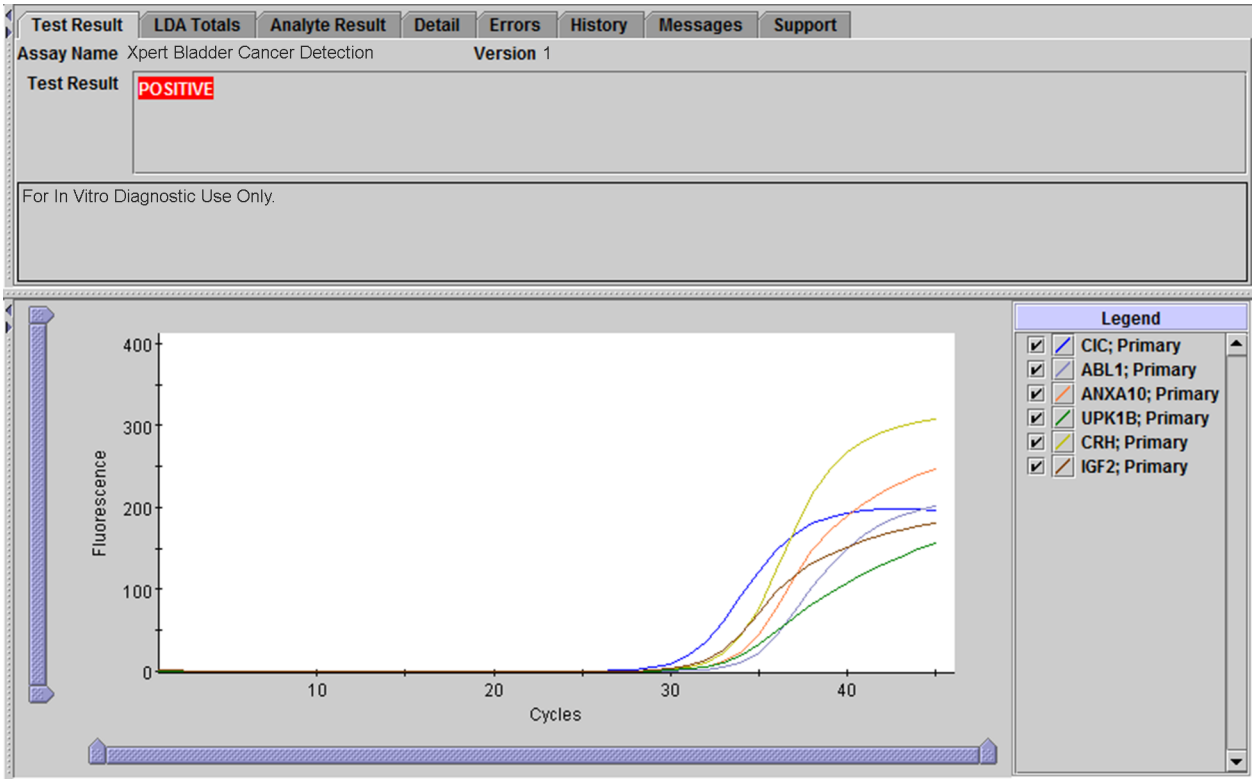


Figure 3. POSITIVE RESULT

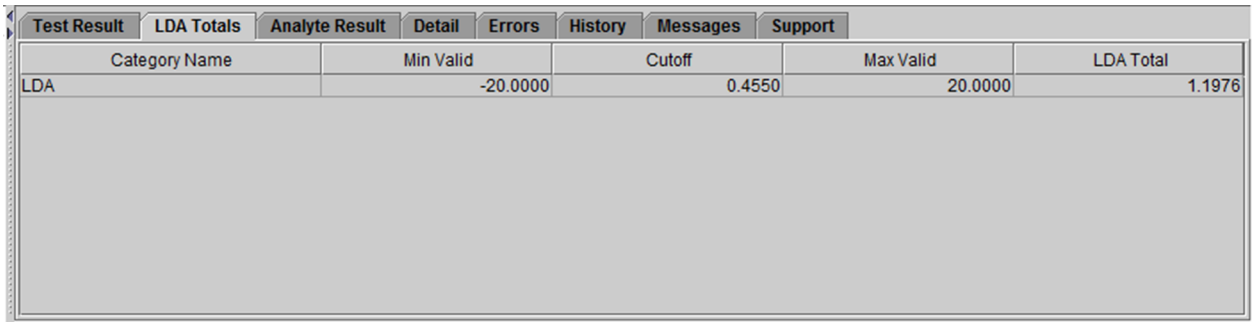


Figure 4. LDA Total – POSITIVE RESULT

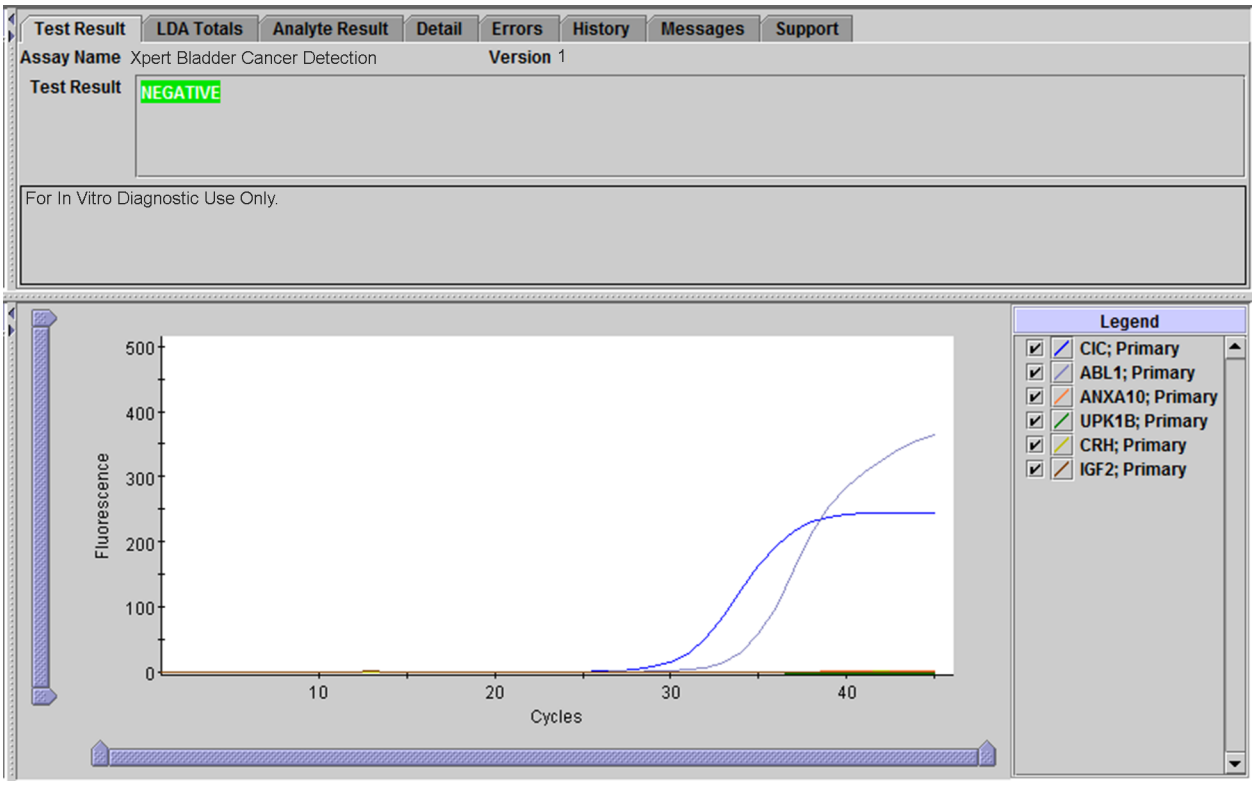
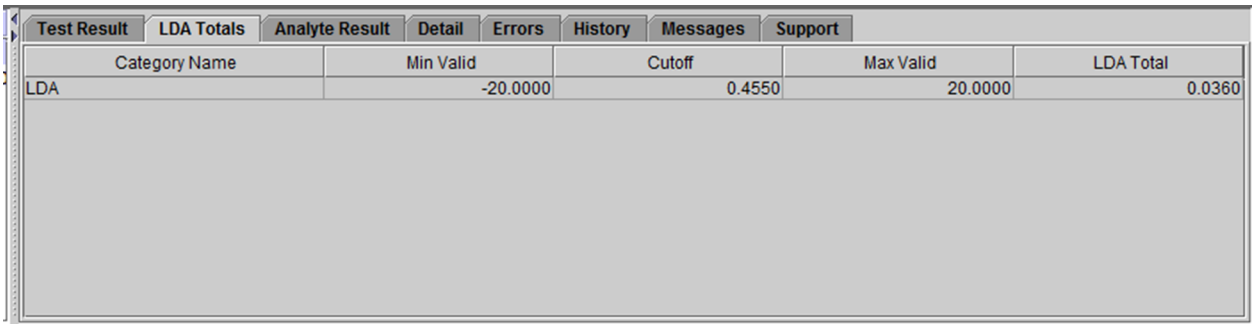


Figure 5. NEGATIVE RESULT



Category Name	Min Valid	Cutoff	Max Valid	LDA Total
LDA	-20.0000	0.4550	20.0000	0.0360

Figure 6. LDA Total – NEGATIVE RESULT

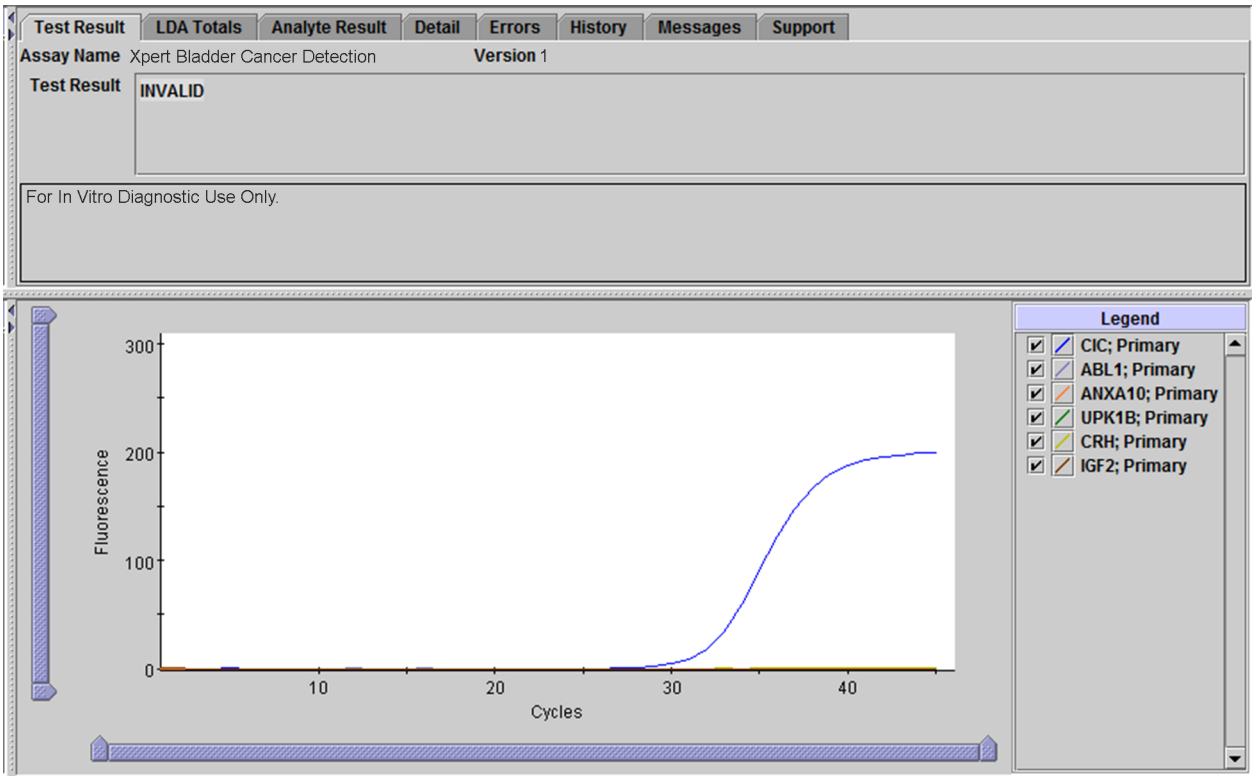


Figure 7. INVALID

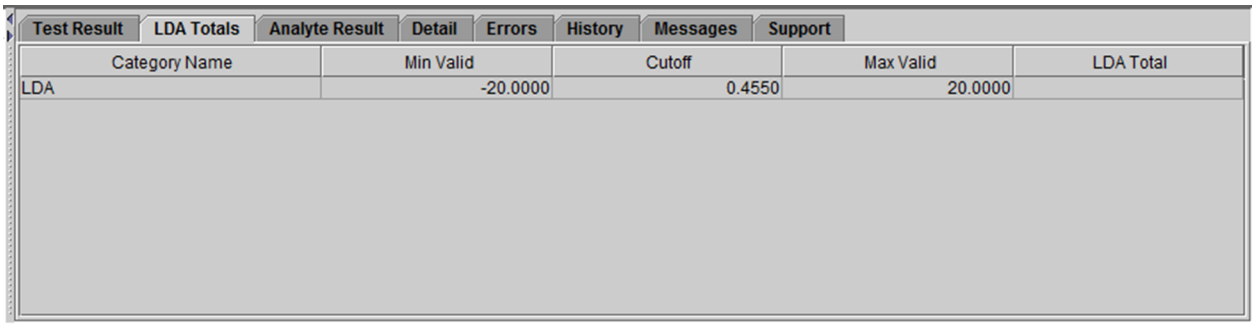


Figure 8. LDA Total – INVALID

16 Retests

16.1 Retest Procedure

For retest of a **NO RESULT**, **INVALID**, or **ERROR** result, use a new cartridge (do not re-use the cartridge).

1. Remove a new cartridge from the kit.
2. See Section 12.1. Preparing the Cartridge, and Section 12.2. Starting the Test.

17 Limitations

- Modifications to these procedures may alter the performance of the test. Results from Xpert Bladder Cancer Detection should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- The performance of Xpert Bladder Cancer Detection was validated using the procedures provided in this package insert only using urine specimens collected from subjects from 19 to 95 years of age.
- Xpert Bladder Cancer Detection detects ABL1, CRH, IGF2, UPK1B and ANXA10 mRNA in voided urine specimens. Diseases and medications that cause increased levels of these mRNAs in urine can result in a positive test result.
- Erroneous test results might occur from improper specimen collection, handling or storage or sample mix-up. Careful compliance to the instructions in this package insert is necessary to avoid erroneous results.
- Assay interference may be observed in the presence of albumin, bilirubin, hemoglobin, whole blood, *Candida albicans, Escherichia coli*, *Pseudomonas aeruginosa*, bacillus Calmette-Guerin (BCG), nitrofurantoin, and phenazopyridine-HCl. The maximum tolerated levels of these substances, as listed in Table 7, are 1.92 g/dL for albumin, 11.25 mg/dL for bilirubin, 0.153 g/dL for hemoglobin, 1% for whole blood, 6e6 cfu/mL for *Candida albicans*, 6e5 cfu/mL for *Escherichia coli*, 6e7 cfu/mL for *Pseudomonas aeruginosa*, 1e6 cfu/mL for BCG, 60mg/dL for nitrofurantoin, and 25.32 mg/dL for phenazopyridine-HCl.
- Mutations or polymorphisms in primer or probe binding regions may result in erroneous but believable results.

18 Expected Values

18.1 Values Among Healthy Volunteers and Urology Referral Subjects

Xpert Bladder Cancer Detection was performed with urine specimens from healthy volunteers and urology referral subjects without symptoms or history of bladder cancer, as part of an assay specificity study (see also Specificity under Clinical Performance below). The distribution of LDA Totals is shown in Figure 9. The assay cut-off is indicated by a blue dotted line.

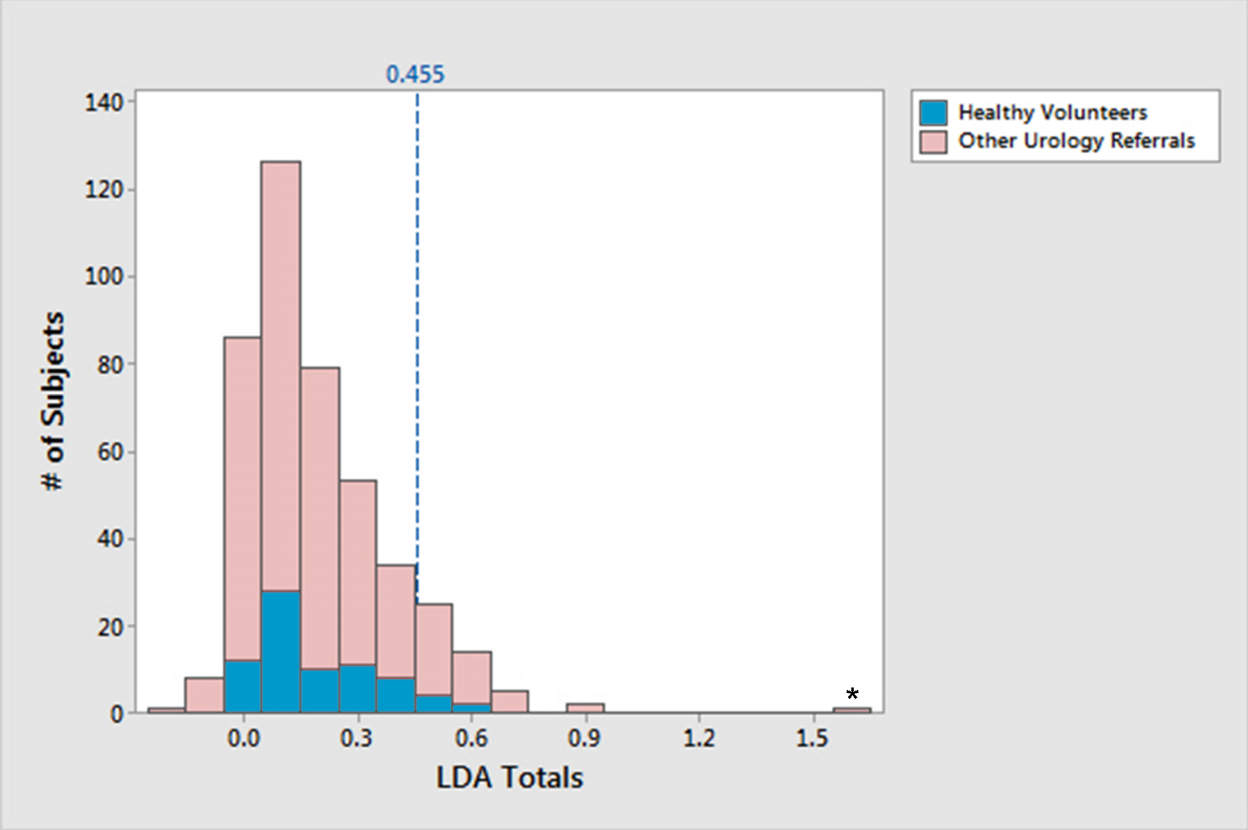


Figure 9. Histogram of Bladder Cancer Detection LDA Totals among Healthy Volunteers and other Urology Referrals

*One subject diagnosed as Bladder cancer presented as maximum LDA Total (Outlier far right)

18.2 Values Among Patients with Symptoms of Bladder Cancer

The distribution of LDA Total results among specimens collected in a prospective study from subjects presenting with symptoms of bladder cancer is shown in Figure 10. (See also Section 19.1. Performance vs. Standard of Care). The distribution is shown for subjects who did (POS) and did not (NEG) have bladder cancer based on cystoscopy and histology results. The assay cut-off is indicated by a blue dotted line.

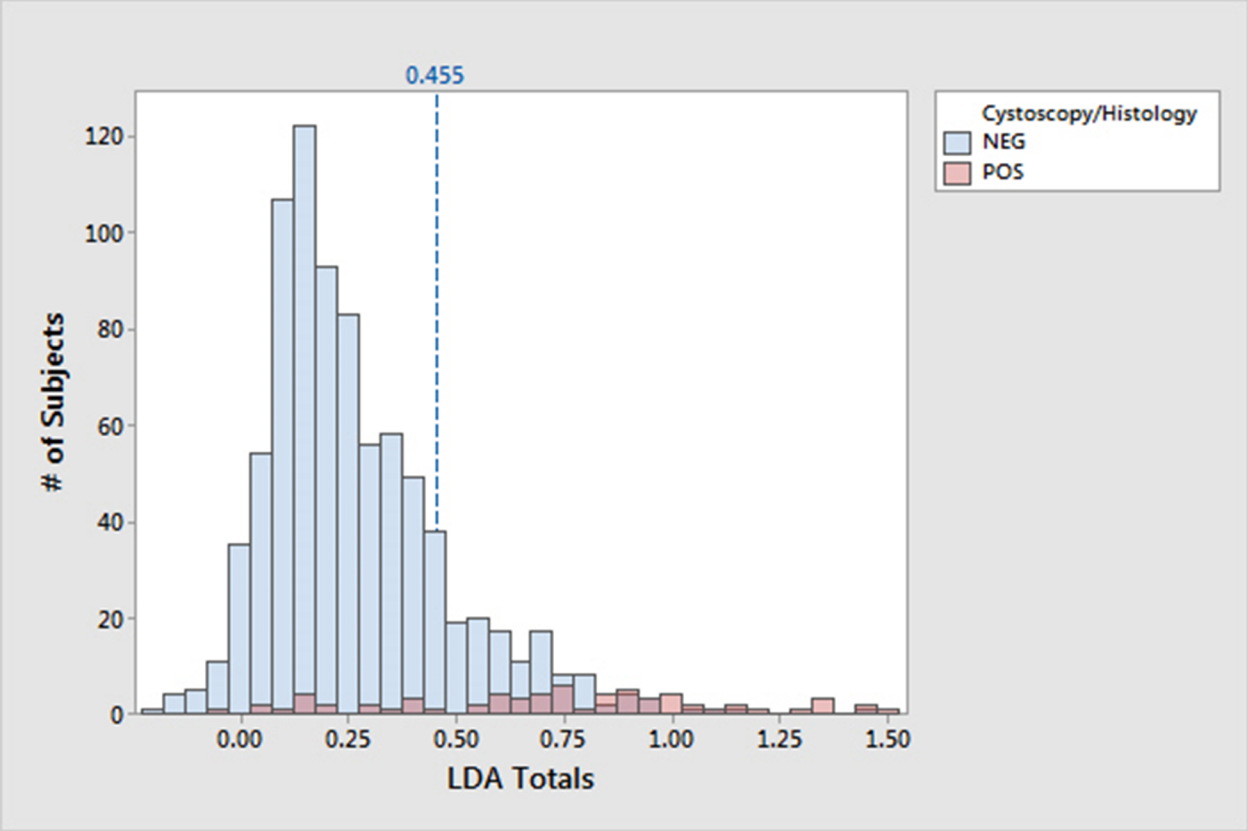


Figure 10. Histogram of Xpert Bladder Cancer Detection LDA Totals Among Subjects with Symptoms of Bladder Cancer

19 Performance Characteristics

19.1 Performance vs. Standard of Care

Performance characteristics of Xpert Bladder Cancer Detection were evaluated at sites in the U.S., Canada and E.U. Subjects included individuals presenting with symptoms of bladder cancer. For study purposes, symptomatic patients were defined as those presenting with patient-reported macroscopic (gross) or asymptomatic microhematuria within 12 weeks of enrollment into the study. For eligible subjects, voided urine specimens were collected for testing with Xpert Bladder Cancer Detection. Results of Xpert Bladder Cancer Detection were compared to cystoscopy, with histology confirmation of positive and suspicious cystoscopies. Patients with positive or suspicious cystoscopies but negative histology findings were considered to be negative for bladder cancer. Subjects with positive and/or suspicious cystoscopy results for whom histology was absent were excluded from the analyses. A total of 1124 subjects were initially enrolled in this study, of which 895 were eligible for inclusion and had valid Xpert Bladder Cancer Detection results.

The demographics for the 895 subjects are summarized in Table 2.

Table 2. Demographics Summary — Bladder Cancer Detection Study

Category		N(%)	
Sex	Male	511 (57.1%)	
	Female	384 (42.9%)	
Race	Caucasian	756 (84.5%)	
	Hispanic	37 (4.1%)	
	Black or African American	81 (9.0%)	
	Asian	9 (1.0%)	
	Other	6 (0.7%)	
	Unknown	6 (0.7%)	
Smoking History	Current Smoker	145 (16.2%)	
	Former Smoker	316 (35.3%)	
	Never Smoked	434 (48.5%)	
Hematuria History	Gross Hematuria	487 (54.4%)	
	Asymptomatic Microhematuria	408 (45.6%)	
Age (yrs)			**Mean ± SD (Range)**
	Overall	895	62.3 ± 13.6 (19-95)
	Male	511 (57.1%)	64.8 ± 13.0 (20-95)
	Female	384 (42.9%)	59.0 ± 13.8 (19-88)

Overall, Xpert Bladder Cancer Detection demonstrated a 75.8% sensitivity and 84.6% specificity relative to cystoscopy/histology (Table 3). The sensitivity among high grade tumors was 88.4% [(38/43) 95% CI: 75.5-94.9]. The sensitivity among low grade tumors was 52.2% [(12/23) 95% CI: 33.0-70.8].

Table 3. Xpert Bladder Cancer Detection vs. Cystoscopy/Histology

		Cystoscopy/Histology		
Xpert Bladder		Pos	Neg	Total
	Pos	50	128	178
	Neg	16	701	717
	Total	66	829	895
		Sensitivity	75.8%(95% CI: 64.2-84.5)	
		Specificity	84.6%(95% CI: 81.9-86.9)	
		PPV	28.1%(95% CI: 22.0-35.1)	
		NPV	97.8%(95% CI: 96.4-98.6)	
		Accuracy	83.9%(95% CI: 81.4-86.2)	
		Prevalence	7.4%(95% CI: 5.8-9.3)	

Xpert Bladder Cancer Detection tests for 95.4% (868/910) of the study specimens were successful on the first attempt with an overall non-determinate rate of 4.6%. The indeterminate cases included 19 **INVALID**, 19 **ERROR** result, and four **NO RESULT** outcomes. Thirty-seven of the 42 indeterminate cases were retested, of which 27 yielded valid results upon repeat assay. The overall rate of assay success was 98.4% (895/910).

19.2 Specificity

In addition to the clinical specificity of 84.6% established in the bladder cancer detection study, a multi-center, prospective study was conducted to establish specificity of Xpert Bladder Cancer Detection in healthy volunteers and urology patients without prior history or clinical evidence of bladder cancer. A total of 537 subjects were initially enrolled in this study, of which 508 were eligible for inclusion and had valid Xpert Bladder Cancer Detection results. The patient population is summarized in Table 4.

Table 4. Demographics Summary — Xpert Bladder Cancer Detection Specificity Study

Category		N(%)	
Sex	Male	341 (67.1%)	
	Female	167 (32.9%)	
Smoking History	Current Smoker	52 (10.2%)	
	Former Smoker	196 (38.6%)	
	Never Smoked	260 (51.2%)	
Cancer History	History of GU[a] Cancer	73 (14.4%)	
	History of non-GU cancer	32 (6.3%)	
	No history of cancer	403 (79.3%)	
Age (yrs)			**Mean ± SD (Range)**
	Overall	508	62.1 ± 15.1 (19-91)
	Male	341 (67.1%)	64.5 ± 14.9 (20-91)
	Female	167 (32.9%)	57.2 ± 14.3 (19-89)

[a] GU=Genitourinary

The overall specificity of Xpert Bladder Cancer Detection in healthy volunteers and subjects presenting for a urology evaluation without previous history or clinical evidence of bladder cancer was 89.8% (456/508). Specimens from 91.9% (68/74) of the healthy volunteers were negative by the Xpert Bladder Cancer Detection. A summary of the overall specificity and the specificity by group is shown in Table 5.

Table 5. Xpert Bladder Cancer Detection vs. Clinical Status

Group	N	TP	FP	TN	FN	Specificity (%) (95% CI)
Healthy Volunteers	74	NA	6	68	NA	91.9% (83.4-96.2)
Other	434	NA	46	388	NA	89.4% (86.2-92.0)
Combined	508	NA	52	456	NA	89.8% (86.8-92.1)

TP=true positive, FP=false positive, TN=true negative, FN=false negative. Other = subjects referred for a urology consultation with no previous history of bladder cancer or clinical evidence of bladder cancer.

20 Analytical Performance

20.1 Minimum Assay Input

The cell and ABL1 mRNA concentrations, which are highly correlated, vary widely between urine specimens. The ABL1 RT-PCR gives robust results out to cycle 36.0. ABL1 serves as a Sample Adequacy Control (SAC) and the maximum allowable Ct for a valid test result is set at 36.0, defining the minimum assay input. Using this sample adequacy cut-off with 895 voided urine specimens, the non-determinate rate was 4.6%, the sensitivity was 75.8% and the specificity was 84.6% (see Section 19).

The performance of the assay was tested near the sample adequacy cut-off and the minimum cell concentration required to meet the sample adequacy requirement was estimated with two cell lines (SW780, ATCC® CRL-2169 and BE(2)-c, ATCC® CRL-2268). First, blank samples (n=30) were prepared by adding individual urine specimens to equal volumes of Xpert Urine Transport Reagent and filtering to remove endogenous cells. Two replicates were tested with each of two reagent lots for each urine sample. All test results were **INVALID** because ABL1 was not detected, or the Ct was greater than 36.0. The remaining volumes of the blank samples were then pooled and used as diluent for the cell line dilutions.

The minimum cell concentration required to achieve 19 out of 20 replicates with ABL1 Ct equal to or less than 36.0 was estimated using logistic regression and by testing replicates of 20 at a minimum of five concentrations over three days of testing. The study was performed with two different lots of Xpert Bladder Cancer Detection and the results are summarized in Table 6.

Table 6. Minimum Assay Input in Xpert Bladder Cancer Detection

Cell Line	Minimum Assay Input (in Cells/mL Unpreserved Sample) Estimates (Logit) (Lower and Upper 95% Confidence Intervals)	
	Lot 1	Lot 2
SW780	13.4 (11.4-18.7)	12.4 (10.6-16.9)
BE(2)-c	42.6 (34.0-61.9)	22.5 (19.1-30.3)

20.2 Analytical Specificity

The primers and probes in Xpert Bladder Cancer Detection were designed to amplify mRNA and to avoid the amplification of human genomic DNA. To determine whether Xpert Bladder Cancer Detection amplifies and detects human genomic DNA, human genomic DNA (Promega G304A) was introduced directly into the RT-PCR assay at 40 ng/mL, 400 ng/mL and 4 µg/mL. Eight replicates per human genomic DNA concentration and eight controls with no added DNA were tested. The Ct results for all targets (ABL1, ANXA10, UPK1B, CRH, and IGF2) were zero (targets not detected) in all of the replicates. These results demonstrate that no cross-reactivity with human genomic DNA was detected with Xpert Bladder Cancer Detection. The human genomic DNA also did not interfere with the CIC reaction at the concentrations tested.

20.3 Interfering Substances

In a non-clinical study, 26 potentially interfering substances, including three microorganisms that may be present in voided urine specimens were evaluated with Xpert Bladder Cancer Detection.

To determine whether the presence of the potentially interfering substances caused assay interference, eight replicate negative and eight replicate positive samples were tested per substance. Solutions of potentially interfering substances were prepared and tested at concentrations at or above those specified in Table 7, which lists the maximum tolerated concentration for each substance. All individual specimens were preserved by adding them to an equal volume of Xpert Urine Transport Reagent, and then combined to create both negative and positive pools. The substances and organisms were then diluted into the negative and positive pools for testing.

The effect of each potentially interfering substance on positive and negative replicates was evaluated by comparing the LDA Total generated in the presence of the substance to the LDA Total from controls lacking the substance.

Of the 26 potentially interfering substances, the substances or organisms that caused **INVALID** test results were *Pseudomonas aeruginosa* at a concentration of 6e8 cfu/mL and *Candida albicans* at a concentration of 6e7 cfu/mL of urine. Three substances caused **ERROR** test results: Nitrofurantoin (at 75mg/dL), Phenazopyridine-HCl (at 33.75 mg/dL), and Hemoglobin (at 0.77 g/dL), although Hemoglobin did not show errors at higher concentrations (up to 2 g/dL).

Six substances caused statistically significant inhibitory effects on the LDA Total result that were more than two standard deviations away from the control mean: Albumin (at 2.4 g/dL), hemoglobin (at 0.61 g/dL), *Escherichia coli* (at 6e6 cfu/mL), bilirubin (at 15 mg/dL), Bacillus Calmette-Guerin (BCG) (at 5.5e6 cfu/mL), and whole blood (at 1.5%). For these substances, titrations were made and the maximum tolerated concentrations were determined and are listed in Table 7.

Table 7. Substances Tested and Maximum Concentrations Tolerated

	Test Concentration[a]	
Analyte	**(SI Units)**	**(Conv. Units)**
Possible Urine Constituents		
Albumin	30 g/L	1.92 g/dL
Ascorbic acid (Vitamin C)	342 µmol/L	6 mg/dL
Bilirubin (unconjugated)	192.4 µmol/L	11.25 mg/dL
Caffeine	308 µmol/L	598 µg/L
Ethanol	21.7 mmol/L	100 mg/dL
Glucose	6.7 mmol/L	120 mg/dL
Hemoglobin	12 g/L	0.153 g/dL
Leukocytes	n/a	1e5/mL
Uric Acid	0.5 mmol/L	9 mg/dL
Sodium Chloride	128.3 mmol/L	750 mg/dL
Nicotine	6.2 umol/L	100.6 µg/dL
Whole Blood	n/a	1% v/v
Possible Microbial Contaminants		
Candida albicans	n/a	6e6 cfu/mL
Escherichia coli	n/a	6e5 cfu/mL
Pseudomonas aeruginosa	n/a	6e7 cfu/mL
Therapeutic Agents		
Acetaminophen	199 µmol/L	30 µg/mL
Bacillus Calmette-Guerin (BCG)	n/a	1e6 cfu/mL
Doxycycline	67.5 µmol/L	3 mg/dL
Mitomycin C	448.7 µmol/L	15 mg/dL
Acetylsalicylic Acid	3.62 mmol/L	65.2 mg/dL
Thiotepa	1.7 mmol/L	32.6 mg/dL
Ampicillin	152 umol/L	1.72 mg/dL
Doxorubicin-HCl	1.1 mmol/L	64.3 mg/dL
Nitrofurantoin	2.5 mmol/L	60 mg/dL
Phenazopyridine-HCl	1.0 mmol/L	25.32 mg/dL
Trimethoprim	2.1 mmol/L	60 mg/dL

[a] **Concentrations for unpreserved (neat) urine**

20.4 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 positive bladder cancer sample. The high positive sample consisted of the cell lines SW780 (ATCC® CRL-2169) at 1.5e5 cells/mL, and BE(2)-c (ATCC® CRL-2268) at 2.5e4 cells/ml, in a 50% Xpert Urine Transport Reagent and 50% synthetic urine background. The testing scheme was repeated 43 times using a single GeneXpert module for a total of 21 high positive and 22 negative samples. All 21 high positive samples were correctly reported as **POSITIVE**. Twenty-one of the negative samples were correctly reported as **NEGATIVE**, and one negative sample was reported as **INVALID** because the Ct value of the CIC was outside the valid range.

20.5 Assay Reproducibility

Reproducibility of Xpert Bladder Cancer Detection was evaluated using a panel of five samples prepared in a background matrix of 50% Xpert Urine Transport Reagent and 50% urine, and spanning the LDA reportable range. Two operators at each of the three study sites tested one panel of five samples over nine testing days (five samples x nine days x two operators x two replicates x three sites). Three lots of Xpert Bladder Cancer Detection cartridges were used at each of the three testing sites. The Xpert Bladder Cancer Detection was performed according to the Xpert Bladder Cancer Detection procedure.

The reproducibility of the Xpert Bladder Cancer Detection was evaluated in terms of the LDA Totals for each sample, relative to expected values. 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 8.

Table 8. Summary of Reproducibility Data

Expected LDA Total	Actual (95% CI)	N	Site/Inst		Lot		Day		Operator/ run		Within-run		Total	
			SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	(%)[a]	SD	CV
0.02	0.02 (-0.02, 0.06)	108	0.00	0.00	0.02	79.4	0.00	0.0	0.00	0.0	0.01	20.6	0.02	0.89
0.31	0.33 (0.14, 0.52)	108	0.00	0.00	0.06	38.8	0.03	11.0	0.01	1.0	0.07	49.1	0.10	0.30
0.55	0.58 (0.43, 0.73)	108	0.00	0.00	0.06	63.5	0.01	2.4	0.00	0.0	0.04	34.1	0.07	0.13
0.54	0.63 (0.43, 0.83)	108	0.00	0.00	0.07	47.7	0.00	0.0	0.04	15.5	0.06	36.8	0.10	0.16
1.24	1.25 (1.08, 1.42)	108	0.00	0.00	0.08	76.4	0.01	1.3	0.00	0.0	0.04	22.3	0.09	0.07

a. (%) is contribution of variance component to total CV.

21 References

1. Burger M et al, Epidemiology and Risk Factors of Urothelial Bladder Cancer. Eur Urol 63 (2013) 234-241.
2. Siegel R, Miller K, Jemal A. Cancer Statistics, 2015. CA: Cancer J Clin, 2015, 65(1): 5-29.
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.

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23 Technical Assistance

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

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Contact information for all Cepheid Technical Support offices is available on our website: https://www.cepheid.com/en/CustomerSupport.

24 Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	CE marking – European Conformity
	Do not reuse

Symbol	Meaning
	Caution
	Expiration date
	Batch code
	Consult instructions for use
	Warning
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Control
	Temperature limitation
	Authorized Representative in the European Community
	Biological risks
	Authorized Representative in Switzerland
	Importer

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IVD CE

25 Revision History

Section	Description of Change
Table of Symbols	Added CH REP and Importer symbols and definitions to Table of Symbols. Added CH REP and Importer information with Switzerland address.
Revision History	Updated revision history table.