GeneXpert
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GeneXpert® Carba-R

REF **RCARBAR-10**

Cepheid

Research Use Only RUO

301-2436, Rev. 2 December 2013

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089-1189
USA

Phone: +1.408.541.4191
Fax: +1.408.541.4192

GeneXpert® Carba-R

For Research Use Only. Not for Use in Diagnostic Procedures.

1. Proprietary Name

GeneXpert® Carba-R

2. Common or Usual Name

Carba-R Assay

3. Summary

The Cepheid GeneXpert® Carba-R Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* test designed for rapid detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{OXA-48,}$ and bla_{IMP-1} gene sequences associated with carbapenem-non-susceptibility in Gram-negative bacteria.

4. Principle of the Procedure

The GeneXpert (GX) Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR assays. 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, 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*.

The primers and probes in the GeneXpert Carba-R Assay detect proprietary sequences for the bla_{KPC} (KPC), bla_{NDM} (NDM), bla_{VIM} (VIM), bla_{OXA-48} (OXA-48), and bla_{IMP-1} (IMP-1) gene sequences associated with carbapenem-non-susceptibility in Gram-negative bacteria. Rectal swabs are collected using the Cepheid collection kit and transported to the testing laboratory. Material on the swab is eluted by breaking one swab into a vial containing Cepheid Sample Reagent followed by vortexing. The eluate is transferred to the sample chamber of the cartridge using the disposable transfer pipette provided in the GeneXpert Carba-R Assay kit. All reagents required for sample preparation and real time PCR analysis are preloaded in the cartridge. Bacterial cells in the eluate are mixed with the sample preparation control and treatment reagents, cells are captured on a filter and lysed by sonication. The released DNA is eluted, mixed with dry PCR reagent, and the solution is transferred to the reaction tube for real-time PCR and detection. Time to result is approximately 48 minutes.

5. Reagents and Instruments

5.1 Material Provided

The GeneXpert Carba-R Assay kit contains sufficient reagents to process 10 samples. The kit contains the following:

GeneXpert Carba-R Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Reagent 1	3 mL per cartridge
• Reagent 2:	
• Guanidinium chloride	2.5 mL per cartridge
GeneXpert Carba-R Assay Sample Reagent Vials	**10**
• Sample Reagent	**1 x 5.0 mL per vial**
Disposable (1.7 mL) Transfer Pipettes	**10**
CD	**1**

Note Safety Data Sheets (SDS) are available at http://www.cepheid.com/tests-and-reagents/literature/msds or http://www.cepheidinternational.com/tests-and-reagents/literature/msds.

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. The manufacturing of the BSA is also performed 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 comingling of the material with other animal materials.

5.2 Storage and Handling

- Store the GeneXpert Carba-R Assay cartridges and reagents at 2 – 28 °C.
- Do not open a cartridge until you are ready to perform testing.
- Do not use any reagents that have become cloudy or discolored.
- Do not use a cartridge that has leaked.

5.3 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert Instrument, computer with proprietary GeneXpert Software Version 4.3 or higher, barcode scanner, and operator manual.
- Specimen Collection Device: Cepheid Catalog number 900-0370
- Printer: If a printer is required, contact Cepheid Technical support to arrange for the purchase of a recommended printer.
- Vortex mixer

6. Warnings and Precautions

- Treat all biological samples, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which samples might be infectious, all biological samples should be treated with universal precautions.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute GeneXpert Carba-R Assay Sample Reagent with other reagents.
- Do not open the GeneXpert Carba-R Assay cartridge lid except when adding sample eluted from the swab.
- Do not shake or drop the cartridge after opening the cartridge lid. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use GeneXpert Carba-R Assay cartridge is used to process one test. Do not reuse spent cartridges.
- Do not open a cartridge kit until you are ready to perform testing.
- Reagent 2 contains Guanidinium chloride (H302, H315 H319), which causes skin and eye irritation.

7. GeneXpert Carba-R Assay Procedure

7.1 Preparing the Cartridge

Important **Place the cartridge into the GeneXpert instrument within 15 minutes of adding the sample into the cartridge.**

To add the sample into the cartridge:

1. Remove the cartridge and Sample Reagent from the kit.
2. Open one vial of the Sample Reagent provided and place one swab into the vial.
3. Replace the unused swab into the transport tube and store at 2-8 °C.
4. Break the swab in the vial at the score mark on the swab and discard the upper portion.
5. Close the Sample Reagent cap and vortex at high speed for 10 seconds.
6. Open the cartridge lid. Using the transfer pipette provided, aspirate the Sample Reagent up to the mark on the pipette (which is approximately 1.7 mL) and then transfer the material into the sample chamber of the GeneXpert Carba-R cartridge. See Figure 1. Retain the remaining sample at 2–8 °C in case a retest is required.
7. Close the cartridge lid.

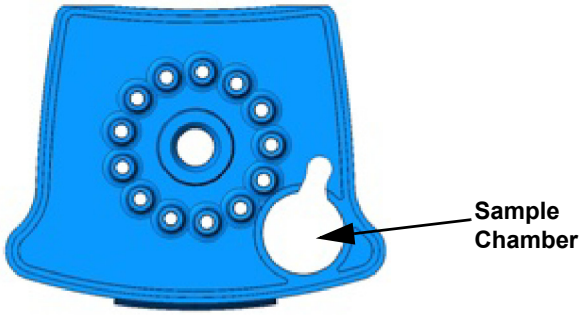

Figure 1. GeneXpert Carba-R Assay Cartridge (Top View)

7.2 Starting the Test

Important **Before starting the test, make sure the GeneXpert Carba-R Assay definition file is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual* depending upon the model that is being used.

1. Turn on the GeneXpert instrument:
   - 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 GeneXpert 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.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** and **Order Test** (Infinity). The Create Test window opens.
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan the barcode on the GeneXpert Carba-R Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the GeneXpert Carba-R cartridge does not scan, then set up a new test by following the retest procedure in section 9.2.

6. 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.
7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). In the dialog box that appears, type your password.
8. 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. Then remove the cartridge.

   D. The used cartridges should be disposed of in the appropriate specimen waste containers according to your institution's standard practices.

7.3 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.*

8. Quality Control

CONTROL **Built-in Quality Controls**

Each test includes a Sample Processing Control (SPC) and a Probe Check Control (PCC)

- **Sample Processing Control (SPC)** – Ensures the sample was correctly processed. The SPC contains spores of *Bacillus globigii* in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that lysis of bacteria has occurred if the organisms are present and verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Probe Check Control (PCC)** – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity, and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

9. Interpretation of Results

9. The results are interpreted by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. Screenshots and interpretations for all possible combinations of results with the five target analytes in the GeneXpert Carba-R assay are not shown; however, the following examples are indicative of the type of results that can be expected.

Note The following table and figures show only representative examples of the types of results that can be expected with the GeneXpert Carba-R Assay. Not all possible combinations of results with the five target analytes are shown.

Result	Interpretation
IMP DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA NOT DETECTED (Figure 2)	IMP target DNA sequence is detected; VIM, NDM, KPC and OXA target DNA sequences are not detected. • PCR amplification of the IMP target DNA gives a Ct value within the valid range and a fluorescence endpoint above the minimum setting; VIM, NDM, KPC, and OXA target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP target DNA amplification can compete with this control. • PCC: PASS; all probe check results pass.
IMP NOT DETECTED; VIM DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA NOT DETECTED (Figure 3)	VIM target DNA sequence is detected; IMP, NDM, KPC, and OXA target DNA sequences are not detected. • PCR amplification of the VIM target DNA gives a Ct value within the valid range and a fluorescence endpoint above the minimum setting; IMP, NDM, KPC, and OXA target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because VIM target DNA amplification can compete with this control. • PCC: PASS; all probe check results pass.

Result	Interpretation
IMP NOT DETECTED; VIM DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA NOT DETECTED (Figure 4)	VIM and NDM target DNA sequences are detected; IMP, KPC, and OXA target DNA sequences are not detected. • PCR amplification of the VIM and NDM target DNAs give Ct values within the valid ranges and fluorescence endpoints above the minimum settings; IMP, KPC, and OXA target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because VIM and NDM target DNA amplifications can compete with this control. • PCC: PASS; all probe check results pass.
IMP DETECTED; VIM NOT DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA NOT DETECTED (Figure 5)	IMP and NDM target DNA sequences are detected; VIM, KPC, and OXA target DNA sequences are not detected. • PCR amplification of the IMP and NDM target DNAs give Ct values within the valid ranges and fluorescence endpoints above the minimum settings; VIM, KPC, and OXA target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP and NDM target DNA amplifications can compete with this control. • PCC: PASS; all probe check results pass.
IMP DETECTED; VIM DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA DETECTED (Figure 6)	IMP, VIM, and OXA target DNA sequences are detected; NDM and KPC target DNA sequences are not detected. • PCR amplification of the IMP, VIM, and OXA target DNAs give Ct values within the valid ranges and fluorescence endpoints above the minimum settings; KPC and NDM target DNA sequences are absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP, VIM, and OXA target DNA amplifications can compete with this control. • PCC: PASS; all probe check results pass.
IMP DETECTED; VIM DETECTED; NDM DETECTED; KPC NOT DETECTED; OXA DETECTED (Figure 7)	IMP, VIM, NDM, and OXA target DNA sequences are detected; KPC target DNA sequence is not detected. • PCR amplification of the IMP, VIM, NDM, and OXA target DNAs give Ct values within the valid ranges and fluorescence endpoints above the minimum settings; KPC target DNA sequence is absent or below the assay detection level. • SPC: Not applicable. The SPC is ignored because IMP, VIM, NDM, and OXA target DNA amplifications can compete with this control. • PCC: PASS; all probe check results pass.

Result	Interpretation
IMP DETECTED; VIM DETECTED; NDM DETECTED; KPC DETECTED; OXA DETECTED (Figure 8)	IMP, VIM, NDM, KPC, and OXA target DNA sequences are detected. • PCR amplification of the IMP, VIM, NDM, KPC, and OXA target DNAs give Ct values within the valid ranges and fluorescence endpoints above the minimum settings. • SPC: Not applicable. The SPC is ignored because IMP, VIM, NDM, KPC, and OXA target DNA amplifications can compete with this control. • PCC: PASS; all probe check results pass.
IMP NOT DETECTED; VIM NOT DETECTED; NDM NOT DETECTED; KPC NOT DETECTED; OXA NOT DETECTED (Figure 9)	IMP, VIM, NDM, KPC, and OXA target DNA sequences are not detected. • IMP, VIM, NDM, KPC, and OXA target DNA sequences are absent or below the assay detection level. • SPC: PASS; PCR amplification of the SPC DNA sequence gives a Ct value within the valid range and a fluorescence endpoint above the minimum setting. • PCC: PASS; all probe check results pass.
INVALID (Figure 10)	Presence or absence of IMP, VIM, NDM, KPC, and OXA target DNA sequences cannot be determined. Use the instructions in the Retest Procedure section to repeat the test. • SPC: FAIL; No PCR amplification of the SPC DNA sequence or the SPC Ct is not within valid range and the fluorescence endpoint is below minimum setting. • PCC: PASS; all probe check results pass.
ERROR	Presence or absence of IMP, VIM, NDM, KPC, and OXA target DNA sequences cannot be determined. Use the instructions in the Retest Procedure section to repeat the test. • SPC: NO RESULT • PCC: FAIL*; all or one of the probe check results fail. The PCC probably failed because the reaction tube was filled improperly or a probe integrity problem was detected. * If the probe check passed, the error is caused by a system component failure.
NO RESULT	Presence or absence of IMP, VIM, NDM, KPC, and OXA target DNA sequences cannot be determined. Use the instructions in the Retest Procedure section to repeat the test. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress). • SPC: NO RESULT • PCC: Not applicable

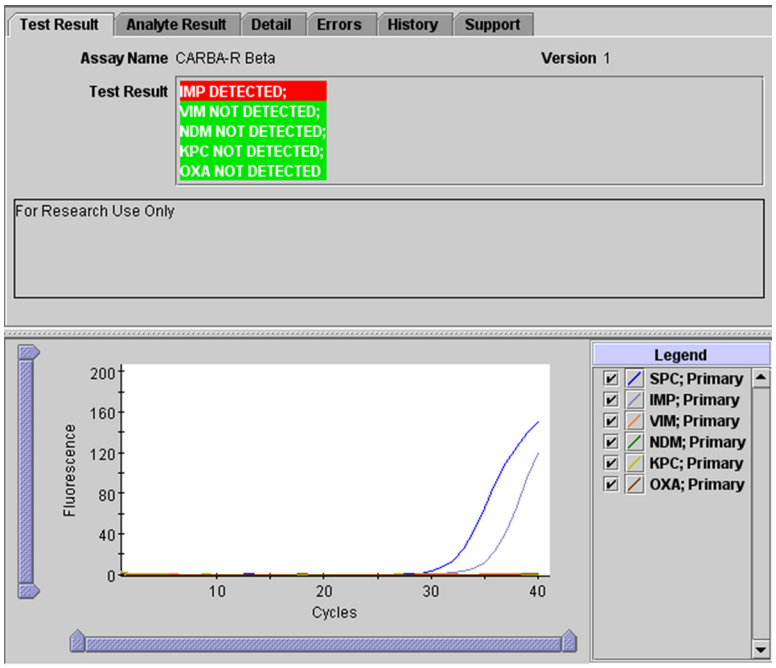

Figure 2. Carba-R Assay - IMP Detected

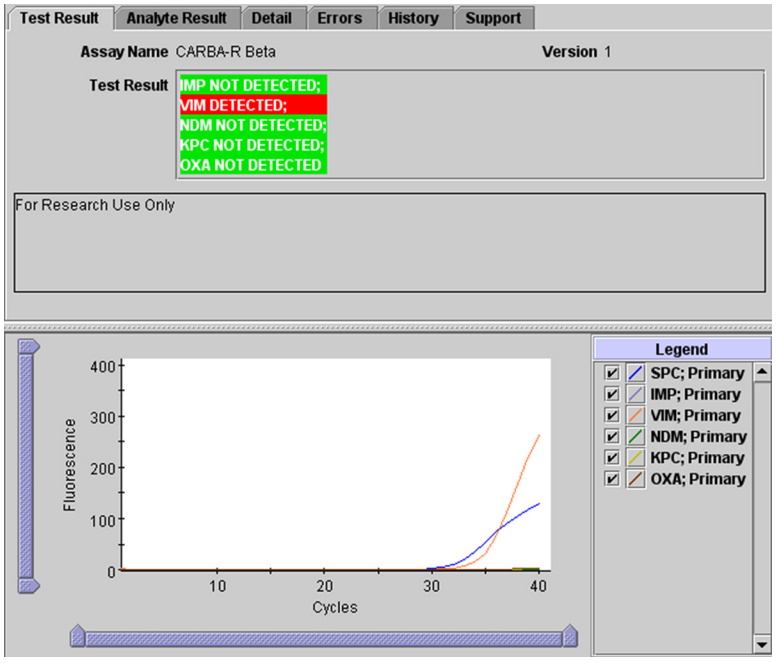

Figure 3. Carba-R Assay - VIM Detected

Note Examples of NDM positive, KPC positive, and OXA positive samples are not shown.

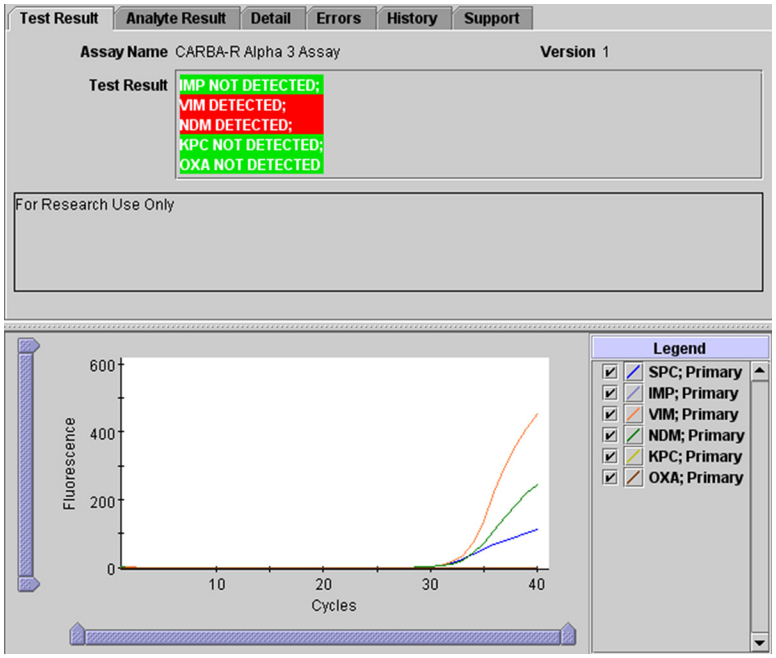

Figure 4. Carba-R Assay - VIM and NDM Detected

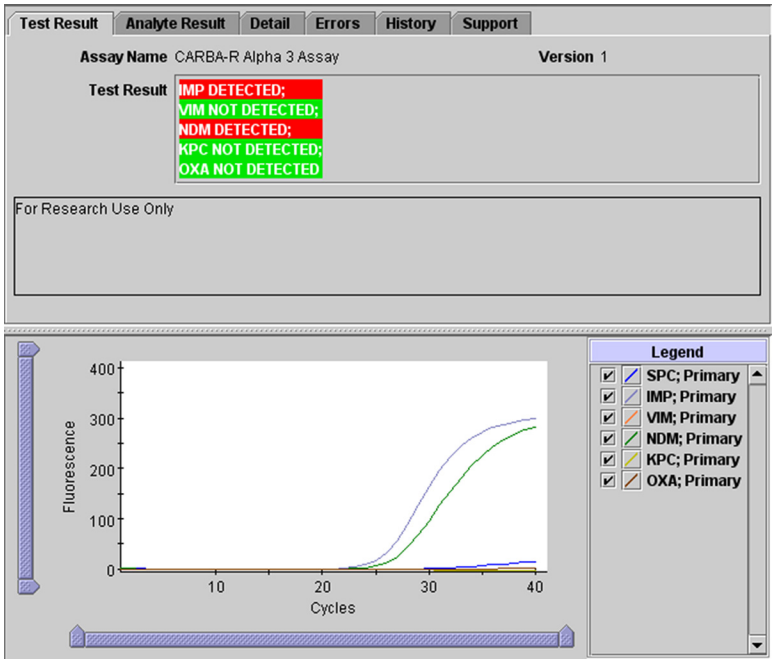

Figure 5. Carba-R Assay - IMP and NDM Detected

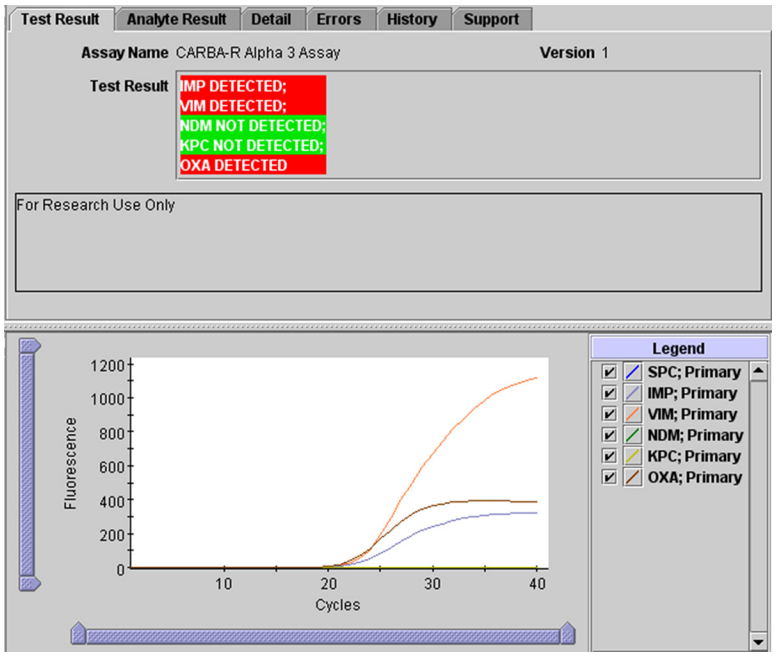

Figure 6. Carba-R Assay – IMP, VIM and OXA Detected

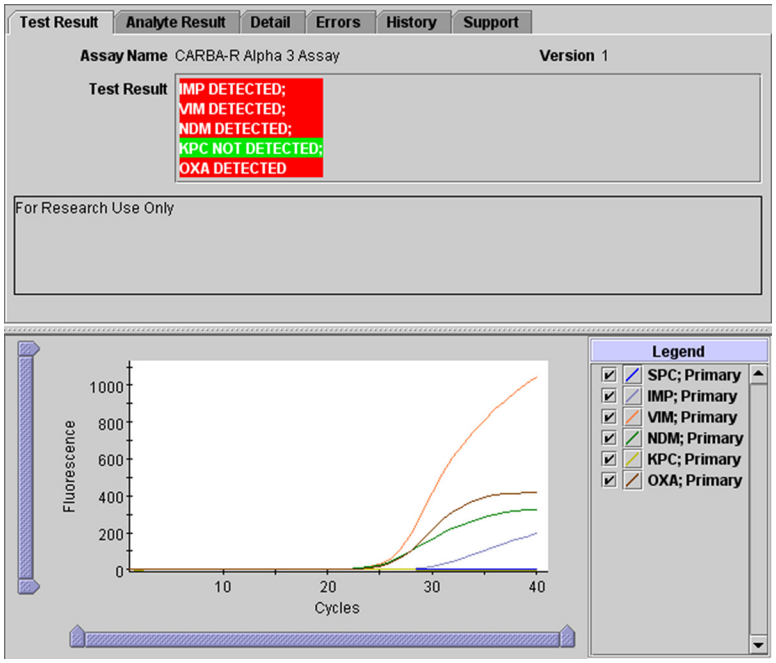

Figure 7. Carba-R Assay – IMP, VIM, NDM, and OXA Detected

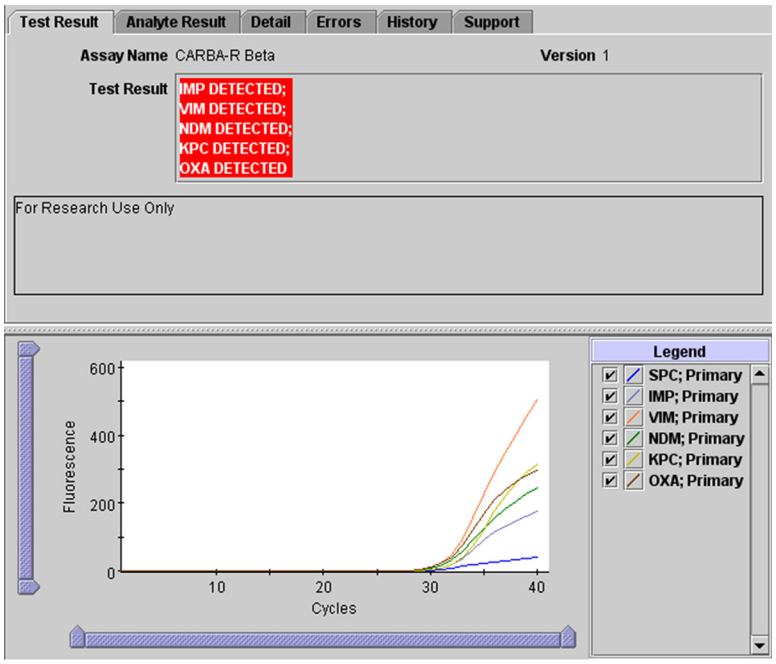

Figure 8. Carba-R Assay – IMP, VIM, NDM, KPC, and OXA Detected

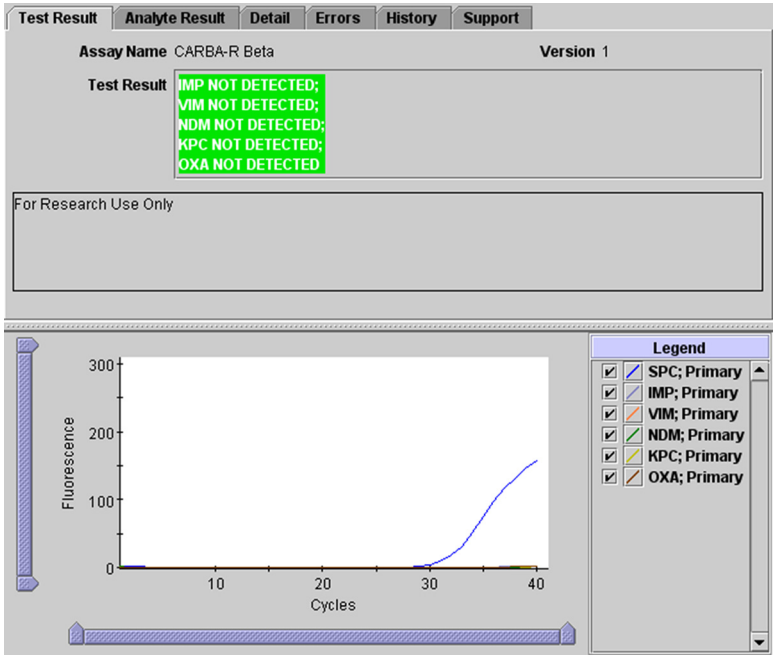

Figure 9. Carba-R Assay – Negative

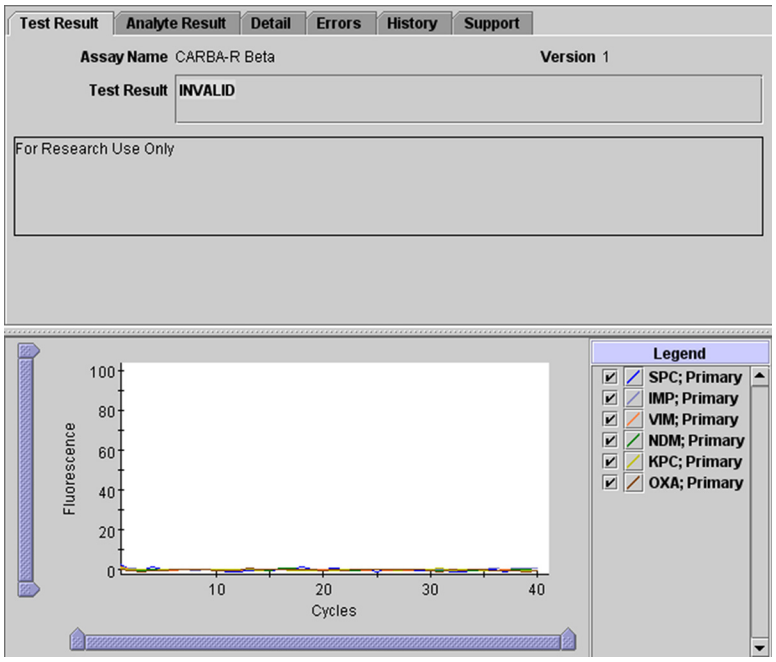

Figure 10. Carba-R Assay – Invalid

9.1 Reasons to Repeat the Test

Repeat the test using a new cartridge (do not re-use the cartridge) and new Sample Reagent vial for dilution.

- An **INVALID** result indicates that the control 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.

9.2 Retest Procedure

1. Remove a new cartridge and new Sample Reagent vial from the kit.
2. Transfer the remaining liquid from the original Sample Reagent containing the vortexed rectal swab sample (that had been stored at 2-8 °C) to the new Sample Reagent vial.
3. Close the Sample Reagent vial cap and vortex at high speed for 10 seconds.
4. Continue with subsequent testing steps starting at step 6 of Section 7.1, Preparing the Cartridge.

10. Limitations

For Research Use Only. Not for Use in Diagnostic Procedures.

11. Performance Characteristics

The performance characteristics of the GeneXpert Carba-R Assay have not been established.

12. Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089-1189 USA	Cepheid Europe S.A.S. Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408.541.4191	Telephone: +33.563.82.53.00
Fax: +1 408.541.4192	Fax: +33.563.82.53.01
www.cepheid.com	www.cepheidinternational.com/

13. 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

Region	Telephone	Email
US	+1 888.838.3222	TechSupport@cepheid.com
France	+33 5 63 82 53 19	Support@cepheideurope.com
Germany	+49 69 50 50 60 647	Support@cepheideurope.com
United Kingdom	+44 3303 332533	Support@cepheideurope.com
South Africa	+27 11 467 7510	Support@cepheideurope.com
Other European, Middle East and African countries	+33 5 63 82 53 19	Support@cepheideurope.com
Other countries not listed above	+1 408.400.8495	TechSupport@cepheid.com

Contact information for other Cepheid offices is available on our website at http://www.cepheid.com/company/contact-us/.

14. Table of Symbols

Symbol	Meaning
	Catalog number
	For Research Use Only. Not for use in diagnostic procedures.
	Do not reuse
	Batch code
	Caution
	Consult instructions for use
	Manufacturer
	Date of Manufacture
	Contains sufficient for <n> tests
	Temperature limitation
	Biological risks

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089-1189
USA

Phone: +1.408.541.4191
Fax: +1.408.541.4192

Dalinis dokumento vertimas iš anglų kalbos

GeneXpert
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Xpert® Carba-R

REF GXCARBAR-CE-10

Cepheid

CE IVD

In Vitro diagnostinė medicinos priemonė

301-2437 Peržiūra B 2014 m. birželio mėn.

In Vitro diagnostinė medicinos priemonė

Patentuotas pavadinimas
Xpert® Carba-R

Bendrinis / įprastinis pavadinimas
Xpert Carba-R tyrimas

Paskirtis
Cepheid Xpert Carba-R tyrimas, atliekamas su GeneXpert® Dx sistema, yra kokybinis in vitro diagnostinis tyrimas, skirtas greitam *la*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48 ir *bla*IMP-1 genų sekų, susijusių su karbapenemams nejautrių gram neigiamose bakterijose, gautų iš rektalinių tepinėlių mėginių, aptikimui ir diferencicijai pacientams, kurie yra žarnyno kolonizacijos su karbapenemams nejautrių bakterijų rizikos grupėje. Tyrime yra naudojama tikro laiko polimerazės grandinės reakcija (PGR). Xpert Carba-R tyrimas yra pagalbinė priemonė karbapenemams nejautrių bakterijų, kolonizuojančių pacientus, aptikimui. Xpert Carba-R tyrimas nėra skirtas karbapenemui jautrių bakterijų infekcijų gydymo stebėjimui ar gydymo skyrimui. Kitos kultūros dėl yra būtinos organizmų atstatymui dėl epidemiologinio tipavimo, antimikrobinio jautrumo tyrimų ir dėl tolimesnių patvirtinančių karbapenemui jautrių bakterijų identifikacinių tyrimų.

Procedūros principas
GeneXpert Dx sistema integruoja ir automatizuoja mėginio apdorojimą, nukleininės rūgšties amplifikaciją bei taikinio eilių aptikimą paprastuose ar kompleksiniuose mėginiuose, naudojant tikro laiko PGR ir RT-PCR tyrimus. Sistemą sudaro instrumentas, personalinis kompiuteris ir įdiegta programinė įranga, skirta mėginių tyrimų paleidimui ir rezultatų peržiūrai. Sistemai yra reikalingos vienkartinio naudojimo kasetės, kuriose yra PGR reagentai ir kuriose vyksta PGR procesas. Kadangi kasetės yra individualios, yra eliminuojamas kryžminis užterštumas tarp mėginių. Dėl pilno sistemos aprašymo prašome žiūrėti *GeneXpert Dx System* naudotojo vadovą.
Xpert Carba-R tyrimo sudėtyje yra reagentų, skirtų *la*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48 ir *bla*IMP-1 genų sekų aptikimui, mėginio apdorojimo kontrolė (SPC) ir mėgintuvėlio tikrinimo kontrolė (PCC). Mėginio apdorojimo kontrolė (SPC) yra skirta adekvataus taikinio bakterijos apdorojimo atlikimui ir inhibitoriaus(-ių) stebėjimui PGR reakcijoje. Mėginio apdorojimo kontrolė (SPC) taip pat užtikrina reakcijos sąlygas (temperatūrą ir laiką), tinkamas amplifikacijos reakcijai ir PGR reagentų veiksmingumui. Tyrimo tikrinimo kontrolė (PCC) patikrina reagento rehidraciją, PGR mėgintuvėlio užpildymą kasetėje, mėgintuvėlio integralumą ir dažų stabilumą.
Pradmenys ir zondai, esantys Xpert Carba-R tyrime, aptinka *bla*KPC (KPC), *bla*NDM (NDM), *bla* VIM (VIM), *bla*OXA-48 (OXA-48) ir *bla*IMP-1 (IMP-1) genų sekas, susijusias su nejautrumu karbapenemui gram neigiamose bakterijose.

Laikymas ir naudojimas

- Xpert Carba-R tyrimo kasetės ir reagentai turi būti laikomi prie 2–28 °C.
- Neatidarykite kasetės tol, kol nebūsite pasiruošę atlikti tyrimo.
- Nenaudokite pasibaigusios galiojimo datos reagentų ar kasečių.
- Mėginio reagentas yra skaidrus bespalvis skystis. Nenaudokite drumzlino ar spalvą pakeitusio mėginio reagento.
- Po atidarymo kasetė turi būti panaudojama per 30 minučių.
- Nenaudokite pratekančios kasetės.

Įspėjimai ir atsargumo priemonės

- Su visais biologiniais mėginiais, įskaitant panaudotas kasetes, elkitės kaip su galinčiais pernešti infekcinius agentus. Kadangi nėra žinoma, kuris mėginys yra infekciškas, su visais biologiniais mėginiais reikia dirbti laikantis universaliųjų atsargumo priemonių.
- Dėl darbo su chemikalais ir biologiniais mėginiais, laikykitės Jūsų laboratorijoje atliekamų saugos procedūrų.
- Dėl tinkamo kasečių ir nepanaudotų reagentų išmetimo pasitarkite su savo įstaigos atliekų utilizavimo skyriumi. Įstaigos turi laikytis savo šalyje galiojančių nurodymų dėl pavojingų atliekų utilizavimo. Ši medžiaga gali demonstruoti pavojingų atliekų charakteristiką – būtina laikytis specifinių utilizavimo taisyklių.
- Rekomenduojama laikytis geros laboratorijos praktikos, įskaitant pirštinių keitimą dirbant su pacientų mėginiais ir mėginių bei reagentų užteršimo vengimo.
- Xpert Carba-R tyrimo reagentų negalima sukeisti su kitais reagentais.
- Neatidarykite Carba-R kasetės dangtelio, išskyrus tą momentą, kai dedate mėginį.
- Nenaudokite kasetės, jei ji po išėmimo iš pakuotės buvo nukritusi.
- Nepurtykite kasetės. Kasetės purtymas ir išmetimas po dangtelio atidarymo gali sukelti klaidingų rezultatų atsiradimą.
- Neklijuokite paciento ID lipduko ant kasetės dangtelio ar brūkšninio kodo etiketės.

- Kiekviena vienkartinio naudojimo Xpert Carba-R tyrimo kasetė yra skirta vieno tyrimo atlikimui. Kasečių nenaudokite pakartotinai.
- Nenaudokite kasetės, kurios reakcijos mėgintuvėlis yra pažeistas.
- Dėvėkite švarų laboratorinį chalatą ir pirštines. Po kiekvieno mėginio apdorojimo keiskite pirštines.
- Darbo vietos ar įrangos užteršimo mėginiais ar kontrolėmis atveju, kruopščiai išvalykite užterštą vietą tirpalu, skiesto santykiu 1:10 buitinio chloro baliklio ir 70% etanolio ar 70% izopropanolio tirpalu. Prieš tęsiant darbą, išvalytą vietą gerai nusausinkite.

- Reagentas 2 savo sudėtyje turi guanidinio chlorino (H302, pavojingas prarijus; H315, dirgina odą; H319, sukelia rimtą akių sudirgimą).

Pagalba
Skambinant ar rašant el. laišką į Cepheid techninės pagalbos skyrių, turėkite šią informaciją:
• Produkto pavadinimas
• Serijos numeris
• Instrumento serijos numeris
• Klaidų pranešimai (jei yra)
• Programinės įrangos versija ir, jei taikoma, kompiuterio serverio numeris.

Europos Sąjunga
Techninis aptarnavimas:
Tel.: +33.563.82.53.19
El. Paštas: techsupport@cepheideurope.fr

Gamintojas
Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
JAV

EC REP Įgaliotas atstovas
Cepheid Europe
Vira Solelh
81470 Maurens-Scopont
France
Tel: +33.563.82.53.00
Fax: +33.563.82.53.01
Email: cepheid@cepheideurope.fr

CE IVD

Tikslus dokumento vertimas į lietuvių kalbą
Vertėjas (-a) A. Gepolevičienė
Data: 2016-10-31
UAB Diamedica
Molėtų pl. 73, Vilnius
Lietuva
Tel. 8 5 279 0080

GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® C. difficile BT

REF **GXCDIFFBT-CE-10**

Cepheid®

***In Vitro* Diagnostic Medical Device** IVD CE

301-6190, Rev. A, June 2016

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Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

Xpert® C. difficile BT

In Vitro Diagnostic Medical Device

1 Proprietary Name

Xpert® *C. difficile* BT

2 Common or Usual Name

Xpert® *C. difficile* BT Assay

3 Intended Use

The Cepheid Xpert *C. difficile* BT Assay, performed on the Cepheid GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test for rapid detection of *C. difficile tcdB* (toxin B gene), *cdt* (binary toxin gene), and a deletion of a nucleotide at position 117 of the *tcdC* gene from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The Xpert *C. difficile* BT Assay is intended as an aid in the diagnosis of CDI and detection of strains potentially associated with more severe disease. The test utilizes automated real-time polymerase chain reaction (PCR) to detect *tcdB*, *cdt*, and the *tcdC* deletion at base 117 associated with the ribotype 027 strain. Binary toxin is produced by a limited number of *C. difficile* strains, including the 027 strain. Binary toxin together with *tcdB* detection is often an indicator of more severe disease or recurrence of disease. Isolates of *C. difficile* that are negative for *tcdB* but contain binary toxin genes alone may produce symptoms similar to toxigenic *C. difficile* strains but the clinical significance of such strains is currently uncertain. Concomitant culture is necessary only if further typing or organism recovery is required.

4 Summary and Explanation

C. difficile is a Gram-positive, spore-forming, anaerobic rod that was first linked to disease in 1978.[1]

CDI ranges from mild diarrhea to severe life-threatening pseudomembranous colitis.[2] Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonization.[3] However, if the normal colonic flora is altered, resistance to colonization by other bacterial species, such as *C. difficile*, is lost. The most common risk factor for developing CDI is exposure to antibiotics.[4] *C. difficile's* primary virulence factor is cytotoxin B.[5] The genes coding for toxin A (*tcdA*; the enterotoxin) and toxin B (*tcdB*) are part of the pathogenicity locus (PaLoc).[6,7] Most pathogenic strains are toxin A-positive, toxin B-positive (A+B+) strains, although toxin A-negative, toxin B-positive (A-B+) variant isolates have been recognized as pathogenic.[8] Some strains of *C. difficile* also produce an actin-specific ADP-ribosyltransferase called CDT or binary toxin. The binary toxin locus contains two separate genes (*cdtA* and *cdtB*) and is located outside the PaLoc.[9-11]

CDI diagnosis traditionally has been based either on the detection of toxin B directly in stool (the cell culture cytotoxicity neutralization [CCCN] test) or on culture of the organism followed by determination of toxin B production by the isolate (toxigenic culture). Both the CCCN test and toxigenic culture are labor intensive but are still considered to be the "gold standards" because of the specificity of the former and the sensitivity of the latter.[12,13] Several rapid enzyme immunoassays have been developed for detection of toxin A and B; however, these tests have reduced sensitivity and specificity compared to the CCCN test. PCR methods for the detection of genes associated with toxin A and/or toxin B production have been developed and show high sensitivity and specificity as compared to toxigenic culture.[14]

In addition to toxin A and B, recent literature suggests a link between the production of binary toxin and both disease severity and outcome. Bauer et al.[15] showed the presence of binary toxin genes in toxigenic isolates in 23% of the CDI cases in Europe. Binary toxin produced by *cdt* genes is frequently observed in *C.difficile* strains associated with increased severity of CDI. Binary toxin belongs to the family of ADP-ribosylating toxins and consists of *cdtA* genes, the enzymatic ADP-ribosyltransferase, which modifies actin, and *cdtB*, which binds to host cells and translocates the product of *cdtA* into the cytosol. Multiple clinical studies indicate an association between the presence of binary toxin genes in *C. difficile* and increased 30-day CDI mortality independent of PCR ribotype. There is also literature showing that subjects having severe CDI, fulminant colitis, and/or recurrent CDI are infected more frequently with *C. difficile* ribotypes carrying the genes for binary toxin production (*cdtA/cdtB*) than those without these complications.[16,17]

A subset of binary-producing isolates have mutations in the negative toxin regulator gene (*tcdC*), i.e., a deletion at nucleotide 117 (*tcdC*Δ117) consistent with Ribotype 027 strains. Infection caused by 027/NAP1/BI strains may be associated with a higher rate of mortality and morbidity, including intensive care unit (ICU)-admission and prolonged length of stay. Multivariate analysis demonstrated a significant association between disease severity and the presence of ribotypes carrying the binary toxin gene with or without deletion at nucleotide 117. In the last several years, there have been outbreaks of CDI attributed to a number of emerging "hypervirulent" strains that include fluoroquinoline-resistant strains belonging to PCR ribotype 027, (which are also known as pulsed-field gel electrophoresis group NAP1 and restriction endonuclease assay type BI.)[8,18] Strains of 027 may exhibit increased toxin production, which is attributed to deletions in the regulatory gene *tcdC* and may produce more spores, leading to enhanced persistence in the environment.[19,20] A presumptive positive 027 result may aid in the identification of possible sources of an 027 outbreak.

Finally, additional studies have reported cases of patients with diarrhea and suspected *C. difficile* infection due to toxinotype XI/PCR ribotype 033, or 033-like strains positive for binary toxin but negative for toxin A and B.[21,22] The clinical significance of such binary toxin positive, toxin B-negative strains is not fully understood.

5 Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid purification and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, personal computer, and preloaded software for running the tests on clinical specimens and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold reagents for PCR and host the processes of DNA extraction, amplification, and amplicon detection. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx System Operator Manual* and/or *GeneXpert Infinity System Operator Manual*.

Xpert *C. difficile* BT Assay includes reagents for the detection of toxin producing *C. difficile* and a Sample Processing Control (SPC). The SPC indicates adequate processing of the target bacteria and monitors the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert *C. difficile* BT assay detect sequences in the genes for toxin B (*tcdB*), binary toxin (*cdt*), and the *tcdC*Δ117.

6 Reagents and Instruments

6.1 Material Provided

The Xpert *C. difficile* BT kit contains sufficient reagents to process 10 specimens or quality control samples.

The kit contains the following:

Xpert *C. difficile* BT Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Reagent 1	3.0 mL per cartridge
• Reagent 2 (Sodium Hydroxide)	3.0 mL per cartridge

Xpert *C. difficile* BT Reagent Pouches	**10**
• Sample Reagent (Guanidinium Thiocyanate)	10 x 2.0 mL per pouch
CD	**1 per kit**
• Assay Definition Files (ADF)	
• Instructions to import ADF into software	
• Package Insert	

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

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

6.2 Storage and Handling

- Store the Xpert *C. difficile* BT kit at 2–28 °C.
- Do not use sample reagent or cartridges that have passed the expiration date.
- Do not open the cartridge lid until you are ready to perform testing.
- Do not use sample reagent that has become cloudy or discolored.
- Do not use a cartridge that has leaked.

6.3 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.3 or higher, barcode scanner, and operator manual.
- Printer: If a printer is required, contact Cepheid Sales Representative to arrange for the purchase of a recommended printer.
- Vortex mixer
- Disposable, clean transfer pipettes
- Dry swab for transfer of the specimen, such as the swab found in the Cepheid Sample Collection Device (Cepheid Catalog Number: 900-0370), Cepheid Single-Use Disposable Swab (Cepheid Catalog Number SDPS-120), or the Copan Dual Swab and Transport Systems (139C LQ STUART)

7 Warnings and Precautions

- Treat all biological specimens, including used cartridges and reagents, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.[23,24]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- Do not substitute Xpert *C. difficile* BT reagents with other reagents.
- Do not open the Xpert *C. difficile* BT cartridge lid except when adding sample and reagents or to remove sample from the original cartridge to perform a retest in a new cartridge.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Do not place the Sample ID label on the cartridge lid or on the barcode label.

- Each single-use Xpert *C. difficile* BT cartridge is used to process one test. Do not reuse a used cartridges.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges and used reagents. This material may exhibit characteristics of hazardous waste requiring specific disposal requirements. Institutions should check their local and country hazardous waste disposal requirements.
- In the event of contamination of the work area or equipment with sample or controls, thoroughly clean the contaminated area with a solution of 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% ethanol. Wipe work surfaces dry completely before proceeding.

8 Chemical Hazards[25,26]

8.1 Reagent 2:

- Contains sodium hydroxide
- Signal Word: Warning
- CLP/GHS Hazard Statements: H302: Harmful if swallowed, H315: Causes skin irritation, H319: Causes serious eye irritation

8.2 Sample Reagent:

- Contains guanidinium thiocyanate
- Signal Word: Warning
- CLP/GHS Hazard Statements: H302: Harmful if swallowed, H412: Harmful to aquatic life with long lasting effects, EUH031: Contact with acids liberates toxic gas
- Precautionary Statements:
  - P264: Wash hands thoroughly after handling.
  - P280: Wear protective gloves/eye protection/face protection.
  - P273: Avoid release to the environment.
  - P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
  - P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - P312: Call a POISON CENTER or physician if you feel unwell.
  - P501: Dispose of contents/container to location in accordance with local and regional/national/international regulations.
  - P362: Take off contaminated clothing and wash before reuse.
  - P321: Specific treatment, see supplemental first aid information.
  - P332 + P313: If skin irritation occurs, get medical advice/attention.
  - P337 + P313: If eye irritation persists, get medical advice/attention.

9 Specimen Collection and Transport

1. Collect the unformed stool in a clean container. Follow your institution's guidelines for collecting samples for *C. difficile* testing.
2. Label with Patient ID and send to the laboratory for testing.
3. Store specimen at 2–8 °C. The specimen is stable for up to 5 days when stored at 2–8 °C. Alternatively, specimens can be kept at room temperature (20–30 °C) for up to 24 hours.

10 Procedure

10.1 Preparing the Cartridge

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

To add the sample to the cartridge (Xpert *C. difficile* BT):

1. Remove the cartridge and Sample Reagent from the package.
2. Immerse swab in the unformed stool sample briefly. The swab does not need to be completely soaked.
3. Insert the swab into the tube containing the Sample Reagent.

Note Use sterile gauze to minimize risks of contamination.

4. Hold the swab by the stem near the rim of the tube, lift the swab a few millimeters from the bottom of the tube, and push the stem against the edge of the tube to break it. Make sure the swab is short enough to allow the cap to close tightly.
5. Close the lid and vortex at high speed for 10 seconds.
6. Open the cartridge lid. Using a clean transfer pipette, transfer the entire contents of the Sample Reagent to the Sample Chamber of the Xpert *C. difficile* BT cartridge. See Figure 1.
7. Close the cartridge lid.

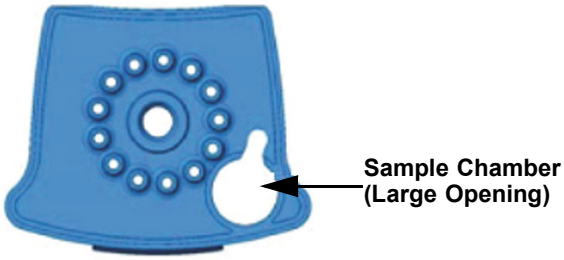

Figure 1. Xpert *C. difficile* BT Cartridge (Top View)

10.2 Starting the Test

Important **Before you start the test, make sure the Xpert *C. difficile* BT assay definition file is imported into the software. This section lists the basic steps of running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.***

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 GeneXpert 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.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (Infinity). The Create Test window opens.
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. 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.

6. Scan the barcode on the Xpert *C. difficile* BT cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert *C. difficile* BT cartridge does not scan, then repeat the test with a new cartridge following the procedure in Section 15, Retest Procedure.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). In the dialog box that appears, type your password.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be loaded automatically, 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 and removing the cartridge.
   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

11 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*.

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.

12 Quality Control

CONTROL

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

- **Sample Processing Control (SPC):** Ensures the sample was correctly processed. The SPC contains spores of *Bacillus globigii* in the form of a dry bead that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that lysis of *C. difficile* bacteria and a spore have occurred if the organisms are present and verifies that specimen processing is adequate. Additionally this control detects sample-associated inhibition of the real-time PCR assay, ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Probe Check Control (PCC):** Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

13 Interpretation of Results

The results are interpreted by the GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. Possible results are shown in Table 1.

Table 1. Xpert *C. difficile* BT Results and Interpretation

Result	Interpretation
Toxigenic C. diff POS, Binary Toxin NEG, 027 NEG See Figure 2.	Toxin-producing *C. difficile* target DNA sequences are detected. • Toxin-producing *C. difficile* — the toxin-producing *C. difficile* target (toxin B gene) has a Ct within the valid range and an endpoint above the minimum setting. • Binary toxin gene and the *tcdC* deletion at nt 117 are not detected. • SPC — NA (not applicable); SPC is ignored since *C. difficile* target amplification may compete with this control • Probe Check — PASS; all probe check results pass.
Toxigenic C. diff POS, Binary Toxin POS, 027 NEG See Figure 3.	Toxin-producing *C. difficile* target DNA sequences are detected. • Toxin-producing *C. difficile* targets (toxin B gene plus binary toxin gene) have Cts within the valid range and endpoints above the minimum setting; the *tcdC* deletion at nt 117 is not detected • SPC — NA (not applicable); SPC is ignored since *C. difficile* target amplification may compete with this control. • Probe Check — PASS; all probe check results pass.
Toxigenic C. diff POS, Binary Toxin POS, 027 PRESUMPTIVE POS See Figure 4.	Toxin-producing *C. difficile* and presumptive 027 target DNA sequences are detected. • All toxin-producing *C. difficile*, presumptive 027 targets (toxin B, binary toxin and *tcdC* deletion at nt 117) have Cts within the valid range and endpoint above the minimum setting. • SPC — NA (not applicable); SPC is ignored since *C. difficile* target amplification may compete with this control. • Probe Check — PASS; all probe check results pass.
Toxigenic C. diff NEG, Binary Toxin POS, 027 NEG See Figure 5.	*C. difficile* toxin B gene sequences are not detected; however, another DNA target (binary toxin gene) is detected and has a Ct within the valid range and an endpoint above the minimum setting. The clinical significance of binary toxin-positive only isolates has yet to be determined. • SPC — NA (not applicable); SPC is ignored since *C. difficile* target amplification may compete with this control. • Probe Check — PASS; all probe check results pass.
Toxigenic C. diff NEG, Binary Toxin NEG, 027 NEG See Figure 6.	*C. difficile* target DNA sequences (Toxin B gene, binary toxin gene) are not detected. • Toxin-producing *C. difficile* gene sequences (toxin B gene and binary toxin gene) are not detected; other DNA targets for toxigenic *C.difficile* (*tcdC* deletion at nt 117) are not detected. • SPC — PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting. • Probe Check — PASS; all probe check results pass.
INVALID See Figure 7.	Presence or absence of *C. difficile* target DNA cannot be determined. Repeat test according to the instructions in Section 15, Retest Procedure. SPC does not meet acceptance criteria, the sample was not properly processed or PCR is inhibited. • INVALID — Presence or absence of *C. difficile* target DNA cannot be determined. • SPC — FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint is below minimum setting. • Probe Check — PASS; all probe check results pass.

Table 1. Xpert *C. difficile* BT Results and Interpretation (Continued)

Result	Interpretation
ERROR	Presence or absence of *C. difficile* target DNA cannot be determined. Repeat test according to the instructions in Section 15, Retest Procedure. The Probe Check control failed probably because the reaction tube was filled improperly, a probe integrity problem was detected, or because the maximum pressure limits were exceeded. • Toxin B — NO RESULT • Binary Toxin — NO RESULT • *tcdC* deletion at nt 117 — NO RESULT • *SPC — NO RESULT • 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 *C. difficile* target DNA cannot be determined. Repeat test according to the instructions in Section 15, Retest Procedure. Insufficient data were collected to produce a test result (for example, the operator stopped a test that was in progress). • Toxin B (*tcdB*) — NO RESULT • Binary Toxin (*cdt*) — NO RESULT • *tcdC*Δ117 — NO RESULT • SPC — NO RESULT • Probe Check — NA (not applicable)

Note The screens shown in this section (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7) are from a GeneXpert Dx instrument running GeneXpert Dx software.

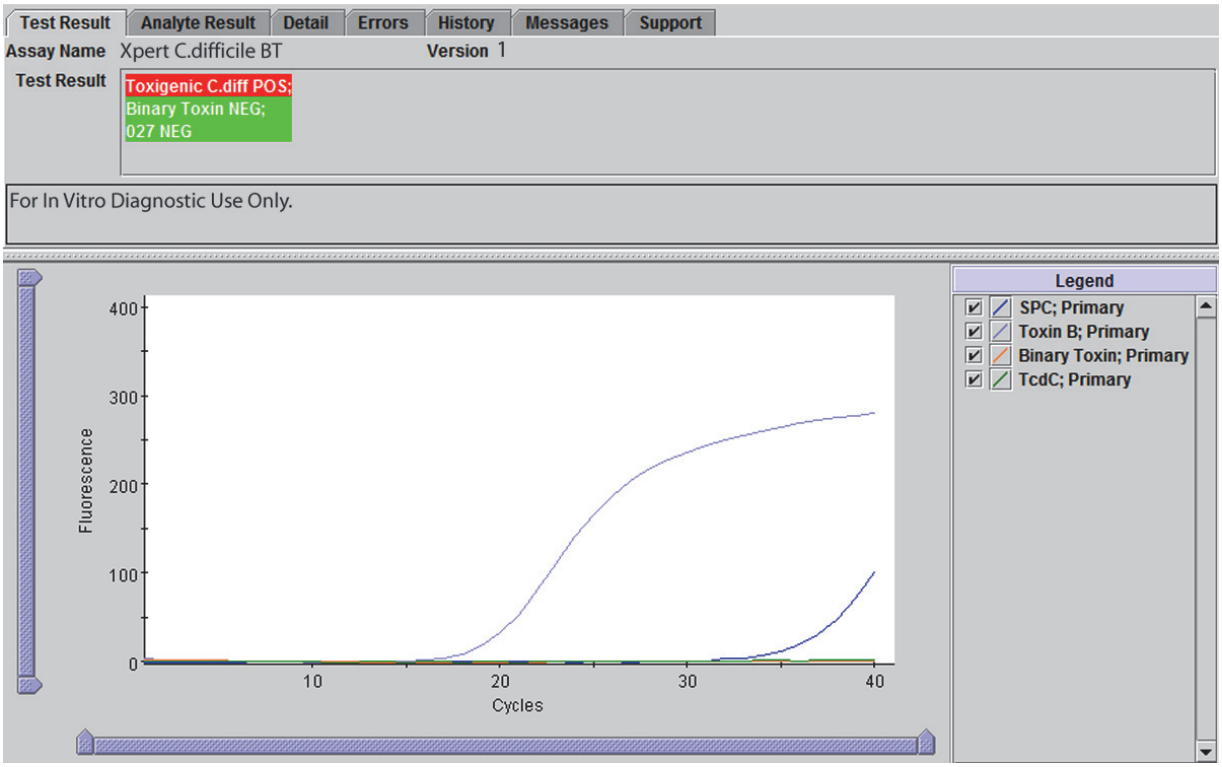

Figure 2. Example of Toxigenic C. diff Positive, Binary Toxin Negative, and 027 Negative Results

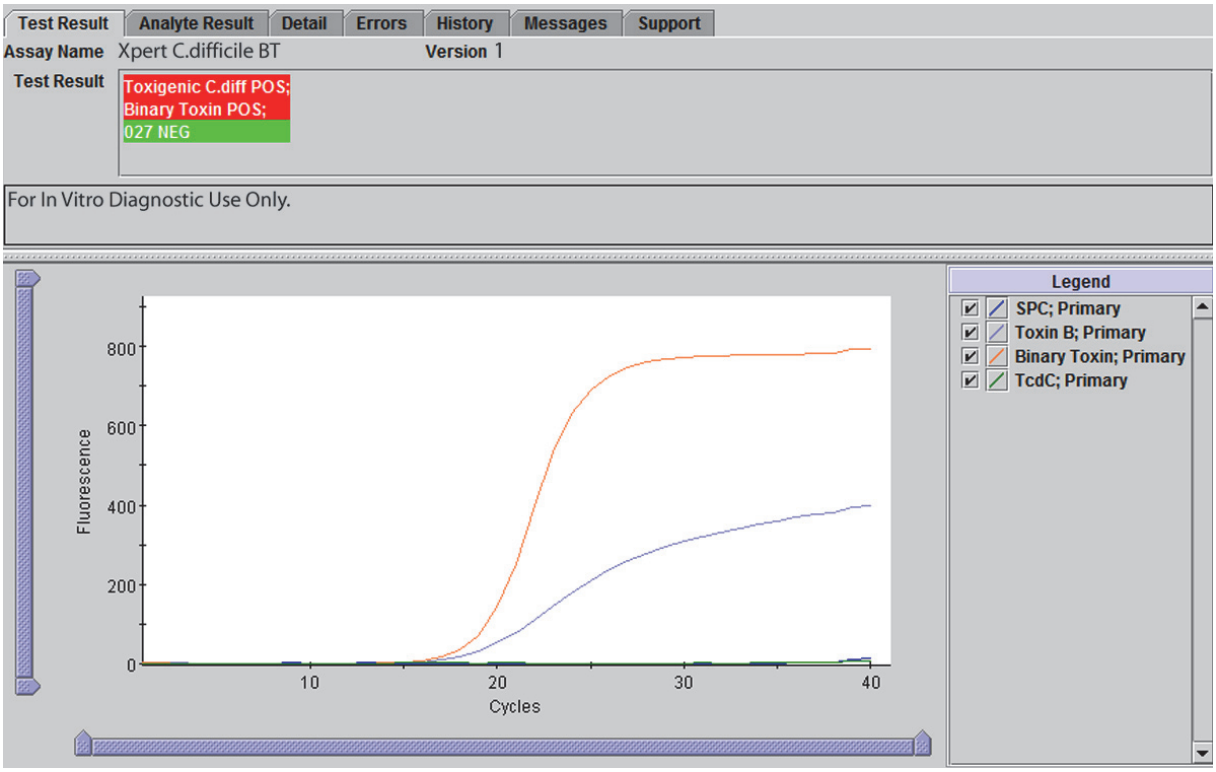

Figure 3. Example of Toxigenic C. diff Positive, Binary Toxin Positive, and 027 Negative Results

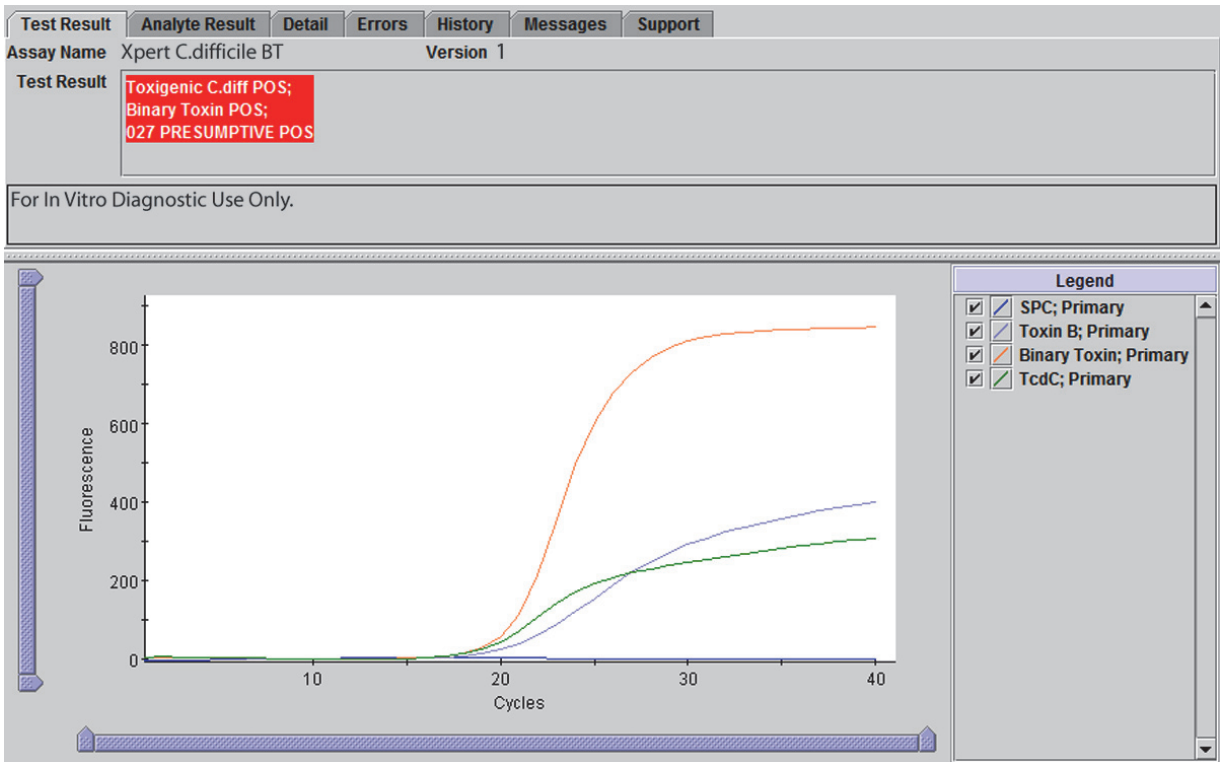

Figure 4. Example of Toxigenic C. diff Positive, Binary Toxin Positive, and 027 Presumptive Positive Results

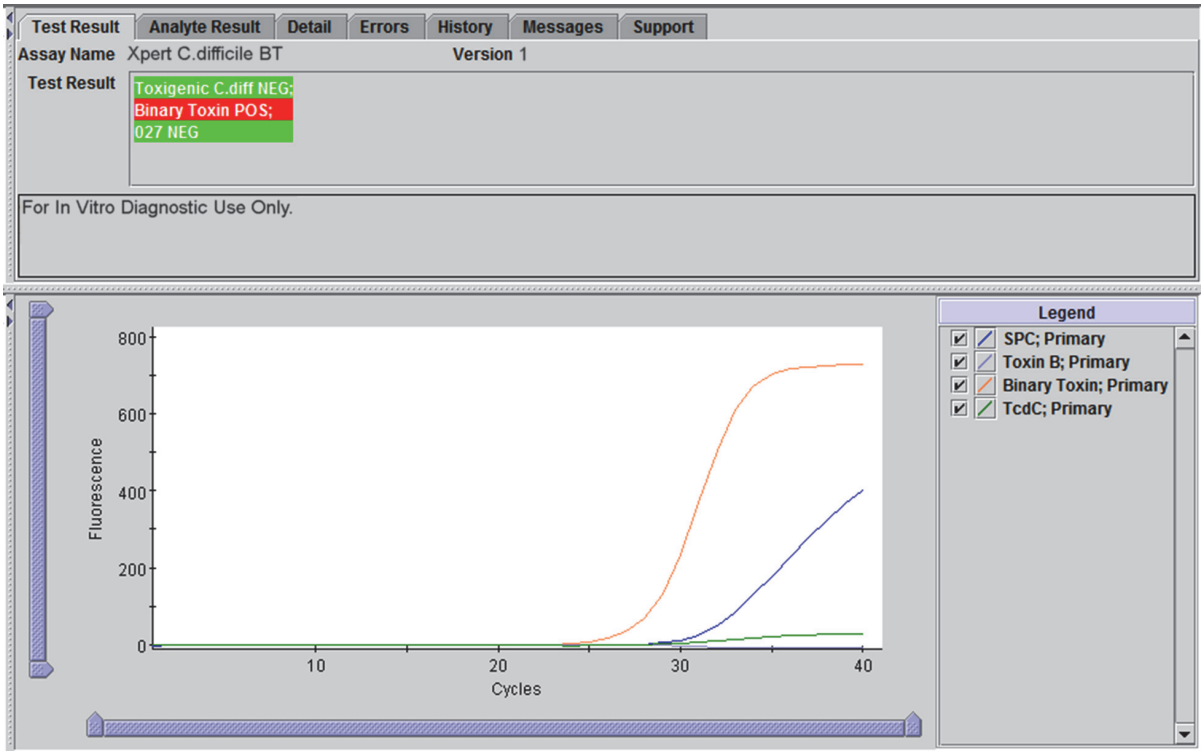

Figure 5. Example of Toxigenic C. diff Negative, Binary Toxin Positive, and 027 Negative Results

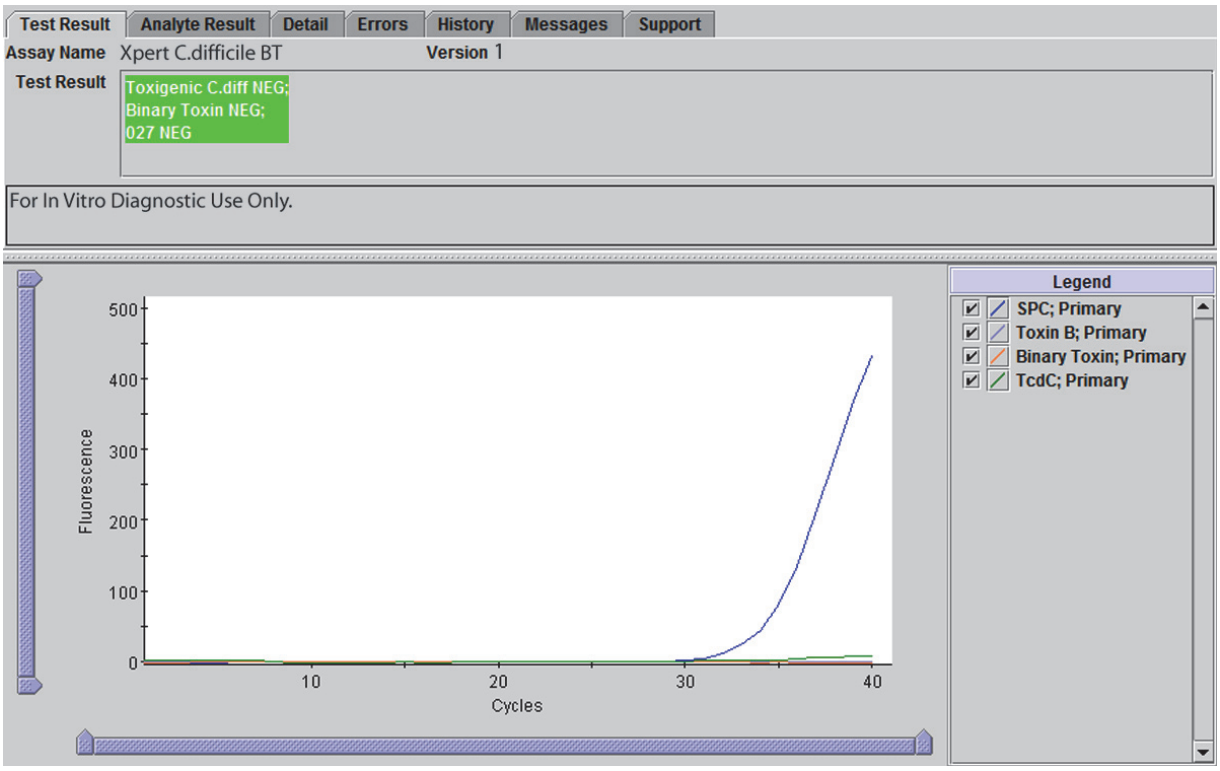

Figure 6. Example of Toxigenic C. diff Negative, Binary Toxin Negative, and 027 Negative Results

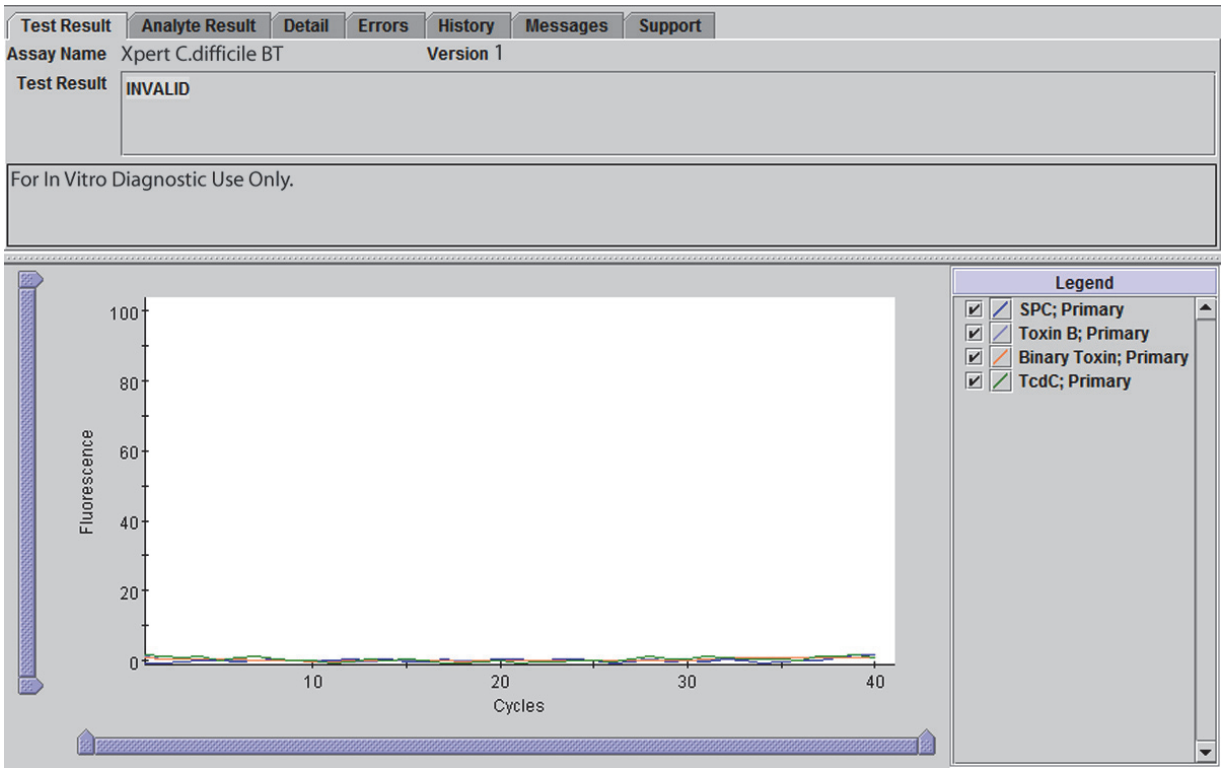

Figure 7. Example of an Invalid Result

14 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test once according to the instructions in Section 15, Retest Procedure.

- An **INVALID** result indicates that the SPC failed. The sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the Probe Check control may have 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, 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.

15 Retest Procedure

For retest within 3 hours of an indeterminate result, use a new cartridge (do not re-use the cartridge) and new reagents.

1. Remove a new cartridge from the kit.
2. Transfer all remaining contents from the Sample Chamber to a new Sample Reagent vial using a disposable transfer pipette.
3. Vortex and add the entire contents of the Sample Reagent to the Sample Chamber of the new Xpert *C. difficile* BT cartridge.
4. Close the lid and start the new test.

For retest after 3 hours of an indeterminate result, repeat the test with a new swab sample from the original patient specimen.

16 Limitations

- Non-027 isolates representing toxinotype XIV will be reported as **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS** using the Xpert *C. difficile* BT Assay.
- **Toxigenic C. diff NEG; Binary Toxin POS, Presumptive 027 NEG** by Xpert *C. difficile* BT may harbor the Toxin B gene and/or the *tcdC* deletion below the LoD of the assay.
- Occasionally, non-027 isolates representing toxinotypes IV, V and X will be reported as **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS** using the Xpert *C. difficile* BT Assay.
- The performance of the Xpert *C. difficile* BT Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert *C. difficile* BT Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because of the dilution factor associated with the retest procedure, it is possible that *C. difficile* positive specimens, very near or at the limit of detection (LoD) of the Xpert *C. difficile* BT Assay, may result in a false negative result upon retest.
- Inhibition of the Xpert *C. difficile* BT Assay has been observed in the presence of the following substances: Zinc oxide paste and Vagisil® cream.
- Outbreaks of CDI may be caused by strains other than 027.
- False-negative results may occur when the infecting organism has genomic mutations, insertions, deletions, or rearrangements or when performed very early in the course of illness.
- Positive results obtained with immunocompromised patients may reflect asymptomatic carriage of *C. difficile*.
- Detection of *C. difficile* nucleic acid in stools confirms the presence of the organisms in patients with diarrhea but may not indicate that *C. difficile* is the cause of the diarrhea.
- Performance characteristics were not established for patients <2 years of age.

17 Expected Values

In the Xpert *C. difficile* BT Assay clinical study, a total of 2293 unformed stool specimens were included from seven centers across the United States and Canada. The number and percentage of toxigenic *C. difficile* positive cases by culture, calculated by age and gender, are presented in Table 2 and Table 3, respectively.

Table 2. Observed Prevalence of Toxigenic *C. difficile* by Age Group[a]

Age Group	N	Toxigenic C. *difficile* Prevalence (includes 027)	Binary Toxin Prevalence	027 Prevalence
2-5	16	37.5% (6/16)	12.5% (2/16)	12.5% (2/16)
6-21	105	12.4% (13/105)	2.9% (3/105)	0.9% (1/105)
22-59	898	16.4% (147/898)	4.8% (43/898)	3.3% (30/898)
>60	1274	20.7% (264/1274)	9.2% (117/1274)	7.2% (92/1274)
Total	2293	18.8% (430/2293)	7.2% (165/2293)	5.5% (125/2293)

a. Prevalence based on Xpert results.

Table 3. Observed Prevalence of Toxigenic *C. difficile* by Gender[a]

Gender	N	Toxigenic C. *difficile* Prevalence (includes 027)	Binary Toxin Prevalence	027 Prevalence
Male	1072	18.2% (195/1072)	6.3% (68/1072)	5.0% (54/1072)
Female	1221	19.2% (235/1221)	7.9% (97/1221)	5.8% (71/1221)
Total	2293	18.8% (430/2293)	7.2% (165/2293)	5.5% (125/2293)

a. Prevalence based on Xpert results.

18 Performance Characteristics

18.1 Clinical Performance

Performance characteristics of the Xpert *C. difficile* BT Assay were determined in a multi-site prospective investigation study at seven US and Canadian institutions by comparing the Xpert *C. difficile* BT Assay to reference culture followed by CCCN testing on the isolates and strain typing on the toxigenic strains by PCR-ribotyping.

Subjects included individuals whose routine care called for *C. difficile* testing. A portion of each leftover unformed stool specimen was obtained for testing by the Xpert *C. difficile* BT Assay. The remaining excess specimen was sent to a central laboratory for reference culture and cytotoxin B testing. Each stool specimen was inoculated onto pre-reduced cycloserine-cefoxitin-fructose agar –direct plate (CCFA-D) and cycloserine cefoxitin mannitol broth with taurocholate lysozyme cysteine (CCMB-TAL). After 24 hours the CCMB-TAL was subcultured on to a second CCFA-E plate (CCFA- Enriched). This direct-enriched culture method is referred to hereafter as “reference culture”.

If *C. difficile* was isolated from the CCFA-D plate and the isolate was positive by CCCN assay, the specimen was classified as “toxigenic *C. difficile* positive” and CCFA-E plate was not further analyzed. If no *C. difficile* was isolated from the CCFA-D plate or if the isolate was negative by cell CCCN assay, the CCFA-E plate was further analyzed.

If CCFA-E was positive for *C. difficile* and the isolate was positive for CCCN assay, the specimen was classified as “toxigenic *C. difficile* positive”. The specimen was reported as “negative” if CCFA-E was negative for *C. difficile* or the isolate was found to be negative by the CCCN assay.

Following reference culture testing, the toxigenic *C. difficile* positive isolates were sent to a second set of reference laboratories for strain identification by PCR-ribotyping.

Performance of the Xpert *C. difficile* BT Assay was calculated relative to the results of direct culture with strain typing and reference culture with strain typing.

18.2 Overall Results

A total of 2293 specimens were tested by Xpert *C. difficile* BT Assay, culture, and strain typing.

Performance Results vs. Direct Culture

Relative to direct culture with PCR-ribotyping, the Xpert *C. difficile* BT Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.78% and 90.86%, respectively. The Xpert *C. difficile* BT Assay also demonstrated a 100% positive agreement and 97.70% negative agreement for 027 (Table 4).

Table 4. Xpert *C. difficile* BT Assay Performance vs. Direct Culture and PCR-Ribotyping

		Direct Culture and PCR-Ribotyping			
		Toxin B+ 027 +	**Toxin B+ 027 -**	**NEG**	**Total[a]**
Xpert *C. difficile* BT[b]	**Toxin B+ 027+**	74	4	47	125
	Toxin B+ 027-	0	164	140	304
	NEG	0	3	1860	1863
	Total	74	171	2047	2292
		Toxigenic *C. difficile*		**Toxigenic *C. difficile* / 027**	
		Sensitivity: 98.78% (242/245) Specificity: 90.86% (1860/2047) Accuracy:91.71% (2102/2292) PPV[c]: 56.41% (242/429) NPV[d]:99.84% (1860/1863)		Pos Agreement: 100% (74/74) Neg Agreement: 97.70% (2167/2218) Accuracy: 97.77% (2241/2292) PPV: 59.20% (74/125) NPV: 100% (2218/2218)	

a. One isolate was not typeable due to contamination: this specimen is not included in the performance statistics.
b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on first attempt.
c. Positive predictive value
d. Negative predictive value

Performance vs. Reference Culture

Relative to reference culture with PCR-ribotyping, the Xpert *C. difficile* BT Assay demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 93.39% and 94.02%, respectively. The Xpert *C. difficile* BT Assay also demonstrated a 98.89% positive agreement and 98.36% negative agreement for 027 (Table 5).

Table 5. Xpert *C. difficile* BT Assay Performance vs. Reference Culture and PCR-Ribotyping

		Reference Culture and PCR-Ribotyping			
		Toxin B+ 027 +	**Toxin B+ 027 -**	**NEG**	**Total[a]**
Xpert *C. difficile* BT[b]	**Toxin B+ 027+**	89	5	31	125
	Toxin B+ 027-	0	217	86	303
	NEG	1	21	1841	1863
	Total	90	243	1958	2291
		Toxigenic *C. difficile*		**Toxigenic *C. difficile* / 027**	
		Sensitivity: 93.39% (311/333) Specificity: 94.02% (1841/1958) Accuracy:93.93% (2152/2291) PPV[c]: 72.66% (311/428) NPV[d]: 98.82% (1841/1863)		Pos Agreement: 98.89% (89/90) Neg Agreement: 98.36% (2165/2201) Accuracy: 98.38% (2254/2291) PPV: 71.20% (89/125) NPV: 99.95% (2165/2166)	

a. One isolate was not typeable due to contamination: this specimen is not included in the performance statistics.
b. Xpert results shown are for first or second attempt. Approximately 3.2% of the specimens were indeterminate on first attempt.
c. Positive predictive value
d. Negative predictive value

Summary

Table 6 lists the total number of specimens for each different Test Result out of the 2293 specimens included in the clinical Performance data analysis.

Table 6. Xpert *C. difficile* BT Assay Overall Performance

Test Result	N
Toxigenic C. diff POS; Binary Toxin NEG; O27 NEG	272
Toxigenic C. diff POS; Binary Toxin POS; O27 NEG	36
Toxigenic C. diff POS; Binary Toxin POS; O27 PRESUMPTIVE POS	122
Toxigenic C. diff NEG; Binary Toxin POS; O27 NEG	7[a]
Toxigenic C. diff NEG; Binary Toxin NEG; O27 NEG	1856
Total	**2293**

a. In additional testing, 4 of 7 strains were shown to harbor the toxin B gene.

Antibiotic Usage

Among the 2293 cases included in the main dataset, antibiotic use within the 2 months prior to sample collection was reported for 1630 and no antibiotic use was confirmed for 570; for 93 cases, antibiotic status was unknown. Antibiotic use did not cause a statistically significant difference in assay performance.

19 Analytical Performance

19.1 Analytical Specificity

Fifty-five (55) strains were collected, quantitated and tested using the Xpert *C. difficile* BT Assay. The strains originated from the American Type Culture Collection (ATCC), Culture Collection University of Göteborg (CCUG), German Collection of Microorganisms and Cell Cultures (DSMZ), the Centers for Disease Control and Prevention (CDC), the Institute of Public Health, Maribor, Slovenia and Swedish Institute for Infectious Disease Control (SMI).

Of the bacterial species that were tested, ten (10) non-toxigenic *C. difficile* strains and eleven (11) non-*C. difficile Clostridium* species were included. The organisms tested were identified as either Gram-positive (37) or Gram-negative (18). The organisms were further classified as aerobic (24), anaerobic (29) or microaerobic (2).

Each strain was tested in triplicate at concentrations ranging from $1.1x10^8$ to $2.2x10^{10}$ CFU/swab. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported **Toxigenic C. diff NEG; Binary Toxin NEG; 027 NEG** (Table 7). The analytical specificity was 100%.

An additional series of non-*difficile Clostridium* species were tested to demonstrate the specificity of the binary toxin assay.

Table 7. Binary Toxin Gene Specificity Study Results

Genus	Species	Number Tested	Toxin A/B	Binary Toxin
Clostridium	*aldenense*	2	neg	neg
Clostridium	*aminovalericum-like*	2	neg	neg
Clostridium	*baratii*	2	neg	neg
Clostridium	*bartletti*	1	neg	neg
Clostridium	*bifermentans*	2	neg	neg
Clostridium	*bolteae*	2	neg	neg
Clostridium	*butyricum*	2	neg	neg
Clostridium	*cadaveris*	2	neg	neg
Clostridium	*celerecrescens*	2	neg	neg
Clostridium	*citroniae*	2	neg	neg
Clostridium	*clostridioforme*	2	neg	neg
Clostridium	*cochlearium*	1	neg	neg
Clostridium	*colicanis*	2	neg	neg
Clostridium	*disporicum*	1	neg	neg
Clostridium	*fallax*	2	neg	neg
Clostridium	*glycolicum*	2	neg	neg
Clostridium	*hastiforme*	1	neg	neg
Clostridium	*hathewayi*	2	neg	neg
Clostridium	*hylemonae*	2	neg	neg
Clostridium	*innocuum*	2	neg	neg
Clostridium	*lactatifermentans*	2	neg	neg
Clostridium	*lavalense*	1	neg	neg
Clostridium	*limosum*	2	neg	neg
Clostridium	*mangenotii*	1	neg	neg
Clostridium	*mayombei-like*	1	neg	neg
Clostridium	*novyi*	2	neg	neg
Clostridium	*paraputrificum*	2	neg	neg
Clostridium	*perfringens*	2	neg	neg

Table 7. Binary Toxin Gene Specificity Study Results (Continued)

Genus	Species	Number Tested	Toxin A/B	Binary Toxin
Clostridium	*perfringens Type E*	3	neg	neg
Clostridium	*ramosum*	2	neg	neg
Clostridium	*sardiniense*	1	neg	neg
Clostridium	*scindens*	2	neg	neg
Clostridium	*septicum*	2	neg	neg
Clostridium	*sordellii*	2	neg	neg
Clostridium	species	19	neg	neg
Clostridium	*spiroforme*	1	neg	neg
Clostridium	*sporogenes*	2	neg	neg
Clostridium	*subterminale* group	3	neg	neg
Clostridium	*symbiosum*	2	neg	neg
Clostridium	*tertium*	2	neg	neg
Clostridium	*tetani*	1	neg	neg
Clostridium	*xylano/aerotolerans*	1	neg	neg
Clostridium	*difficile* RT 027	5	+	+
Clostridium	*difficile* RT 078	2	+	+

All the non-binary toxin containing isolates were negative with the Xpert *C. difficile* BT Assay.

19.2 Analytical Sensitivity

Studies were performed to determine the 95% confidence intervals for the analytical limit of detection (LoD) of *C. difficile* diluted into a fecal matrix of human origin that can be detected by the Xpert *C. difficile* BT Assay. The fecal matrix consisted of human liquid feces (*C. difficile* negative by Xpert *C. difficile* BT Assay) diluted in PBS with 15% glycerol. The LoD is defined as the lowest number of colony forming units (CFU), per swab that can be reproducibly distinguished from negative samples with 95% confidence.

Replicates of 20 were evaluated at each *C. difficile* concentration tested (CFU/swab) for 7 different *C. difficile* strains representing toxinotypes 0 (two strains), III (two strains), IV, V, and VIII (one of each strain).

The estimate and confidence intervals were determined using logistic regression with data (number of positive results per number of replicates at each level) over the range of CFUs tested. The confidence intervals were determined using maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix. The LoD point estimates and 95% upper and lower confidence intervals for each *C. difficile* toxinotype tested are summarized in Table 8.

Table 8. 95% Confidence Intervals for Analytical LoD–*C. difficile*

Strain ID	Toxinotype	$LoD_{95\%}$ (CFU/Swab)	Lower 95% CI	Upper 95% CI
VPI 10463 (CCUG19126)	0	255	190	632
90556-M6S (ATCC9689)	0	460	419	587
LUMC-1 (027)[a]	III	23	19	31
LUMC-5 (027)[a]	III	75	45	176
LUMC-7	V	45	34	104
LUMC-6	VIII	60	50	74
9101	XII	41	34	49

a. By PCR-ribotyping

The results of this study indicate that the Xpert *C. difficile* BT Assay will produce a positive *C. difficile* result 95% of the time for a fecal sample containing 460 CFU/swab and an 027 presumptive positive result 95% of the time for a swab containing 75 CFU.

In addition to the LoD determination, eighteen *C. difficile* strains representing toxinotypes 0 plus 12 variant toxinotypes, including four 027 toxinotype III isolates, were tested using the Xpert *C. difficile* BT Assay. *C. difficile* strains were selected to broadly represent the majority of *C. difficile* toxinotypes encountered in practice. Stock cultures were prepared by suspending the bacterial growth from agar plates in PBS buffer containing 15% glycerol. The concentration of each stock was adjusted to 1.4-5.9 McFarland units. All strains were serially diluted to approximately 900 CFU/swab and tested in triplicate.

Under the conditions of this study, the Xpert *C. difficile* BT Assay correctly identified all 18 strains tested as **Toxigenic C. diff POS**. Included in the panel were 8 toxinotypes reported to be positive for binary toxin (CDT) production as well. All were CDT positive using the Xpert *C. difficile* BT Assay. All four 027 isolates representing toxinotype III were correctly identified as **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS**.

Seven *C. difficile* isolates of PCR ribotype 033 and three additional *C. difficile* isolates of related PCR ribotype that were negative for *tcdA* and *tcdB* but produced binary toxin (CDT)[22] were tested with the Xpert *C. difficile* BT Assay. All 10 isolates yielded positive results for binary toxin only (Table 9), confirming the ability of the assay to detect isolates that are Toxin A-, toxin B-, binary toxin +).

Table 9. Testing Organisms that Produce Binary Toxin Only (Toxin A-, Toxin B-) with Xpert *C. difficile* BT Assay

Organism	Strain ID	PCR Ribotype	Test Result
C. difficile	CD12-066	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	CD12-203	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	CD13-022	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	06-08-02	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	06-20-01	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	NT077	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	AI-0016	238	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	WA-0012	239	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	ES-0145	288	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG
C. difficile	R-0010	033	Toxigenic C.diff NEG; Binary Toxin POS; 027 NEG

19.3 Interfering Substances

Twenty-one (21) biological and chemical substances occasionally used or found in stool specimens were tested for interference with the Xpert *C. difficile* BT Assay. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste (see Section 16, Limitations). The 19 substances listed in Table 10 showed no detectable interference with the Xpert *C. difficile* BT Assay.

Table 10. Substances Tested and Showing No Assay Interference

Substance	Substance
Whole Blood Karolinska University Hospital	K-Y Jelly/Gelée® McNeil-PPC
Mucin (porcine) Sigma	Vaseline Unilever
Kaopectate® Chattem	Dulcolax® Boehringer Ingelheim Pharmaceuticals

Table 10. Substances Tested and Showing No Assay Interference (Continued)

Substance	Substance
Immodium® McNeil-PPC	Preparation H Portable Wipes Wyeth Consumer Healthcare
Pepto-Bismol® Proctor & Gamble	Vaginal Contraceptive Film (VCF) Apothecus Pharmaceutical
Preparation H® Wyeth Consumer Healthcare	Vancomicin Fluka
Fleet® CB Fleet Company	Metronidazole Actavis
Fecal fats Karolinska University Hospital	Anusol® Plus TM Warner-Lambert Company
Monistat® McNeil-PPC	E-Z HDTM High Density Barium Sulfate for suspension E-Z EM Canada
Hydrocortisone Cream Longs Drugs	

20 Reproducibility

A panel of 7 specimens with varying concentrations of toxigenic *C. difficile* and *C. difficile* Ribotype 027 were tested on 10 different days by two different operators at each of the three sites (7 specimens x 2 operators/ day x 10 days x 3 sites). One lot of Xpert *C. difficile* BT Assay was used at each of the 3 testing sites. Xpert *C. difficile* BT Assays were performed according to the Xpert *C. difficile* BT Assay procedure. Results are summarized in Table 11 and Table 12.

Table 11. Summary of Reproducibility Results (All)

Specimen ID	% Agreement[a]			% Total Agreement by Sample
	Site 1	Site 2	Site 3	
Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Low Positive	100% (20/20)	85% (17/20)	85% (17/20)	90% (54/60)
Toxigenic *C. difficile* Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Ribotype 027 High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toxigenic *C. difficile* Ribotype 027 Low Positive	100% (20/20)	95% (19/20)	95% (19/20)	96.7% (58/60)
Toxigenic *C. difficile* Ribotype 027 Moderate Positive	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
% Total Agreement by Site	100% (140/140)	97.1% (136/140)	97.1% (136/140)	98.1% (412/420)

a. For negative and high negative samples, % Agreement = (# negative results/total samples run); for low and moderate positive samples, % Agreement = (# positive results/total samples run).

Table 12. Summary of Ct Value Results by Sample Level and Probe

SPC			
Level	**Ave**	**StdDev**	**CV**
Toxigenic *C. diff* high neg	32.17	0.59	1.83%
Toxigenic *C. diff* low pos	32.14	0.53	1.66%
Toxigenic *C. diff* mod pos	31.98	0.47	1.47%
027 high neg	32.11	0.65	2.03%
027 low pos	31.93	0.72	2.26%
027 mod pos	31.96	0.61	1.90%
Neg	32.26	0.72	2.22%
***tcdB* (Toxin B)**			
Level	**Ave**	**StdDev**	**CV**
Toxigenic *C. diff* high neg	39.59	0.70	1.77%
Toxigenic *C. diff* low pos	35.88	0.81	2.24%
Toxigenic *C. diff* mod pos	32.17	0.45	1.39%
027 high neg	39.11	0.98	2.50%
027 low pos	35.49	0.58	1.65%
027 mod pos	32.10	0.63	1.97%

An additional panel of 6 specimens, three negative and three toxigenic *C. difficile* high-negative, were tested on 5 different days by two different operators at each of the three sites (6 specimens x 2 operators/ day x 5 days x 3 sites). The high negative specimens were prepared at a concentration below LoD such that they were expected to give a negative result 20 to 80% of the time. One lot of Xpert *C. difficile* BT Assay was used at each of the 3 testing sites. Xpert *C. difficile* BT Assays were performed according to the Xpert *C. difficile* BT Assay procedure. Results are summarized in Table 13.

Table 13. Summary of Additional Reproducibility Specimen Results

	% Agreement[a]			% Total Agreement by Sample
Specimen ID	**Site 1**	**Site 2**	**Site 3**	
Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Toxigenic *C. difficile* High Negative[b]	60% (18/30)	60% (18/30)	53.3% (16/30)	57.8% (52/90)

a. (# negative results / total high negative samples run)
b. 20-80% agreement expected for high negative sample

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22 Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408.541.4191	Telephone: +33 563 825 300
Fax: +1 408.541.4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

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

Region	Telephone	Email
US – Technical Support	+1 888.838.3222	techsupport@cepheid.com
Australia and New Zealand	1800 107 884 0800 001 028	techsupportanz@cepheid.com
China	+86 021 5406 5387	techsupportchina@cepheid.com
France	+33 563 825 319	support@cepheideurope.com
Germany	+49 69 710 480 480	support@cepheideurope.com
United Kingdom	+44 3303 332 533	support@cepheideurope.com
Italy	+39 800 902 567	support@cepheideurope.com
South Africa	+27 861 22 76 35	support@cepheideurope.com
Other European, Middle East and African countries	+33 563 825 319 +971 4 253 3218	support@cepheideurope.com
Japan	+0120 95 4886	support@japan.cepheid.com
Countries not listed above	+1 408.400.8495	techsupport@cepheid.com

Contact information for other Cepheid offices is available on our website at www.cepheid.com, www.cepheidjapan.com or www.cepheidinternational.com under the **SUPPORT** tab. Select the **Contact Us** option.

24 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
CE	CE marking – European Conformity
EC REP	Authorized Representative in the European Community
°C	Temperature limitation
	Biological risks
	Warning

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

Vertimas iš anglų kalbos

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® C. difficile BT

REF **GXCDIFFBT-CE-10**

Cepheid

CE IVD

In Vitro diagnostinė medicinos priemonė

301-6190 Peržiūra A. 2016 birželio mėn.

Prekybinis pavadinimas, patentai ir autorinės teisės

Cepheid®, Cepheid logotipas, GeneXpert® ir Xpert® yra prekybiniai ženklai, priklausantys Cepheid.
Windows® yra prekybinis ženklas, priklausantis Microsoft Corporation.

ŠIO PRODUKTO ĮSIGIJIMAS SUTEIKIA NEPERLEIDŽIAMĄ TEISĘ NAUDOTI PRODUKTĄ, LAIKANTIS PAKUOTĖS APRAŠYME PATEIKTŲ NURODYMŲ. JOKIOS KITOS TEISĖS NĖRA SUTEIKIAMOS NEI NETIESIOGIAI, NEI ESTOPPEL PRINCIPU. BE TO, ŠIO PRODUKTO ĮSIGIJIMAS NESUTEIKIA TESIĖS JĮ PERPARDUOTI.

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

Xpert® C. difficile BT

In Vitro diagnostinė medicinos priemonė

1. Patentuotas pavadinimas
Xpert® *C. difficile* BT

2. Bendrinis / įprastinis pavadinimas
Xpert *C. difficile* BT tyrimas

Paskirtis
Cepheid Xpert *C. difficile* BT tyrimas, naudojamas su Cepheid GeneXpert® instrumentų sistemomis, yra kokybinis *in vitro* diagnostinis tyrimas, skirtas greitam *C. difficile tcdB* (toksino B geno) *cdt* (binarinio toksino geno) ir *tedC* geno 117 pozicijos nukleotido delecijos nustatymui išmatų mėginiuose, surinktuose iš pacientų, įtariamų dėl *Clostridium difficile* infekcijos (CDI). Xpert *C. difficile* BT tyrimas yra naudojamas kaip pagalbinė priemonė diagnozuojant CDI ir padermių, potencialiai susijusių su sunkesniais susirgimais, aptikime. Tyrime yra naudojama automatizuota tikro laiko polimerazės grandinės reakcija (PGR) *tcdB*, *cdt*, ir *tcdC* delecijos 117 bazėje, susijusioje su 027 ribotipo paderme, aptikimui. Binarinis toksinas yra produkuojamas riboto skaičiaus *C. difficile* padermių, įskaitant 027 padermę. Binarinis toksinas kartu su *tcdB* aptikimu yra dažnas daug sunkesnių susirgimų ar ligos atsinaujinimo indikatorius. *C. difficile* izoliatai, neigiami dėl *tcdB,* bet turintys toksino genų, gali sukelti simptomus, panašius į toksigeniškos *C. difficile* padermės, tačiau klinikinė tokių padermių svarba kol kas nėra žinoma. Jei yra reikalingas tolimesnis tipavimas, būtina atlikti lydinčiuosius tyrimus.

4. Santrauka ir paaiškinimas
C. difficile yra Gram teigiama, sporas formuojanti anaerobinė bakterija, pirmą kartą su susirgimu susieta 1978m.[1]
CDI sukelia susirgimus, pradedant nuo diarėjos baigiant ūmiu gyvybei pavojingu pseudomembraniniu kolitu.[2] Subrendusi gaubtinės žarnos bakterinė flora suaugusiame žmoguje paprastai yra atspari *C. difficile* kolonizacijai.[3] Tačiau, kai normali gaubtinės žarnos flora susilpnėja, atsparumas kolonizacijai dingsta. Dažniausias rizikos faktorius yra antibiotikų vartojimas.[4] Pirminis *C. Difficile* virulentiškumo faktorius yra citotoksinas B.[5]
Genai, koduojantys toksiną A (*tcdA*; enterotoksinas) ir toksiną B (*tcdB*) yra patogeniškumą koduojančio lokuso dalis (PaLoc).[6,7] Dauguma patogeninių padermių yra teigiamos toksinui A, toksinui B (A+B+), nors toksinui A neigiami ir toksinui B teigiami (A-B+) variantų izoliatai buvo pripažinti kaip patogeniniai.[8] Kai kurios *C. difficile* padermės taip pat produkuoja aktinui specifišką ADP-ribosiltransferazę, vadinamą CDT arba binariniu toksinu. Binarinio toksino lokusas susideda iš dviejų genų (*cdtA* ir *cdtB*) ir yra už PaLoc ribų.[9-11]
CDI diagnozė tradiciškai yra paremta arba toksino B aptikimu tiesiogiai išmatose (ląstelių kultūros citotoksiškumo neutralizavimo [CCCN] tyrimas), arba organizmo kultūros auginimu ir toksino B produkavimo izoliate nustatymu (toksigeno kultūra). Ir CCCN tyrimas, ir toksigeno kultūros tyrimas reikalauja daug darbo, tačiau vis tiek yra laikomi "auksiniais standartais" dėl pirmojo specifiškumo ir antrojo jautrumo.[12,13] Toksino A ir toksino B aptikimui buvo sukurta keletas greitų imunofermentinių tyrimų, tačiau, šių tyrimų jautrumas ir specifiškumas yra mažesnis, lyginant su CCCN tyrimu. Buvo sukurti PGR metodai, skirti genų, susijusių su toksino A ir/ar toksino B produkavimu, aptikimui, demonstruojantys aukštą jautrumą ir specifiškumą, lyginant su toksigeno kultūra.[14]
Be toksino A ir B, naujausia literatūra pateikia sąsajas ir tarp binarinio toksino produkavimo ir susirgimo sunkumo bei pasekmių. Bauer ir kt.[15] pateikė duomenis apie binarinių toksinų genų

buvimą toksigeniniuose izoliatuose 23% CDI atvejų Europoje. Binarinis toksinas, produkuojamas *cdt* genų, yra dažnai pastebimas *C.difficile* padermėse, susijusiose su sunkia CDI. Binarinis toksinas priklauso ADP-ribozilinančių toksinų šeimai ir susideda iš *cdtA* genų, fermentinės ADP-riboziltransferazės, kuri modifikuoja aktiną, ir *cdtB*, kuris suriša ląsteles-šeimininkes ir translokuoja *cdtA* produktą į citozolį. Klinikinės studijos pateikia sąsają tarp binarinio toksino genų buvimo *C. difficile* ir padidėjusio 30 dienų CDI mirtingumo, nepriklausomai nuo PGR ribotipo. Literatūroje taipogi teigiama, jog subjetai su sunkia CDI, žaibiniu kolitu ir/ar atsinaujinusia CDI, yra dažniau infekuojami *C. difficile* ribotipais su binarinį toksiną (*cdtA/cdtB*) produkuojančiais genais, nei komplikacijų nepatiriantys subjektai.[16,17]
Binarinius toksinus produkuojančių izoliatų poaibis turi mutacijų neigiamo toksino reguliaciniame gene (*tcdC*), t.y., delecija ties nukleotidu 117 (*tcdC*Δ117) atitinka 027 ribotipo padermes. Infekcija, sukelta 027/NAP1/BI padermės, gali būti susijusi su didesniu mirtingumu ir sergamumu, įskaitant slaugymą intensyvios priežiūros palatoje bei prailgintą buvimą ligoninėje. Atlikta daugiamatė analizė demonstravo ženklią sąsają tarp susirgimo sunkumo ir ribotipų su binarinio toksino genu buvimu su ar be delecijos ties 117 nukleotidu. Per pastaruosius keletą metų, buvo užfiksuoti CDI proveržiai, sukelti "hipervirulentiškų" ir fluorokvinolonui atsparių padermių, priklausančių PGR ribotipui 027 (taip pat žinomam kaip pulso lauko gelio elektroforezės grupė NAP1 ir restrikcinės endonukleazės tyrimo tipas). [8,18] 027 padermės gali demonstruoti padidintą toksinų produkavimą, kuris yra priskiriamas prie delecijų gene *tcdC* ir gali produkuoti daugiau sporų, taip sustiprinant išliekamumą aplinkoje.[19,20] Galimas teigiamas 027 rezultatas gali būti pagalbinė priemonė identifikuojant galimus 027 protrūkio šaltinius.
Galiausiai, atlikus papildomus tyrimus, buvo pateikti atvejai, kai pacientams su diarėja buvo įtariama *C. difficile* infekcija dėl toksinotipo XI/PCR ribotipo 033, ar į 033 panašių padermių, teigiamų dėl binarinio toksino, bet neigiamų dėl toksino A ir B.[21,22] Klinikinė šių teigiamų binariniam toksinui ir neigiamų B toksinui padermių svarba dar nėra iki galo išaiškinta.

5. Procedūros principas

GeneXpert Dx sistema integruoja ir automatizuoja mėginio apdorojimą, nukleininės rūgšties išgryninimą ir amplifikaciją bei taikinio eilių aptikimą paprastuose ar kompleksiniuose mėginiuose, naudojant tikro laiko PGR tyrimą. Sistemą sudaro instrumentas, personalinis kompiuteris ir įdiegta programinė įranga, skirta mėginių tyrimų paleidimui ir rezultatų peržiūrai. Sistemai yra reikalingos vienkartinio naudojimo GeneXpert kasetės, kuriose yra PGR reagentai ir kuriose vyksta DNR ekstrakcija, amplifikacija ir amplikono aptikimas. Kadangi kasetės yra individualios, yra eliminuojamas kryžminis užterštumas tarp mėginių. Pilną sistemų aprašymą rasite atitinkamame *GeneXpert Dx sistemos operatoriaus vadove* ir/ar *GeneXpert Infinity sistemos operatoriaus vadove*.
Xpert *C. difficile* BT sudėtyje yra reagentų, skirtų toksino, produkuojančio *C. difficile* aptikimui bei mėginio apdorojimo kontrolė (SPC). SPC yra skirta adekvataus taikinio bakterijos apdorojimo atlikimui ir inhibitorių stebėjimui PGR reakcijoje. Tyrimo tikrinimo kontrolė (PCC) patikrina reagento rehidraciją, PGR mėgintuvėlio užpildymą kasetėje, mėgintuvėlio integralumą ir dažų stabilumą.
Xpert *C. difficile* BT tyrimo pradmenys ir mėgintuvėliai aptinka toksino B (*tcdB*), binarinio toksino B (*tcdB*) ir *tcdC*Δ117 genų sekas.

6. Reagentai ir instrumentai

6.1 Tiekiamos medžiagos

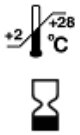

Xpert *C. difficile* BT rinkinį sudaro reagentai, kurių pakanka 10 pacientų ar kokybės kontrolės mėginių.

Rinkinį sudaro:

Xpert *C. difficile* BT tyrimo kasetės su integruotais reakcijų mėgintuvėliais	**10**
• Rutuliukas1, rutuliukas 2 ir rutuliukas 3 (užšaldyti, sausi)	po 1 kasetėje
• Reagentas 1	3.0 mL kasetėje
• Reagentas 2 (natrio hidroksidas)	3.0 mL kasetėje
Xpert *C. difficile* BT reagentų pakuotės	**10**
• Mėginio reagentas (Guanidinio tiocinatas)	1 x 2.0 mL pakuotėje
CD	**1 rinkinyje**
• Tyrimo aprašymo failai (ADF)	
• ADF importavimo į programinę įrangą instrukcijos	
• Pakuotės aprašymas	

Pastaba:
Medžiagos saugos duomenų lapai (MSDL) yra pateikiami www.cepheid.com arba www.cepheidinternational.com skirtuke „SUPPORT“.
Šiame produkte esantis jaučio serumo albuminas (BSA) buvo pagamintas išskirtinai iš jaučio plazmos Jungtinėse Amerikos Valstijose. Gyvūnai nebuvo šeriami ruminantiniais ar kitais gyvulinės kilmės baltymais; gyvūnam buvo atlikti priešmirtiniai ir pomirtiniai tyrimai. Proceso metu medžiaga nebuvo sumaišoma su kitomis gyvūninės kilmės medžiagomis.

6.2 Laikymas ir naudojimas



- Xpert *C. difficile* BT rinkinys turi būti laikomas prie 2–28 °C.
- Nenaudokite pasibaigusios galiojimo datos reagentų ar kasečių.
- Neatidarykite kasetės tol, kol nebūsite pasiruošę atlikti tyrimo.
- Nenaudokite drumzlinų ar spalvą pakeitusių reagentų.
- Nenaudokite pratekančios kasetės.

6.3 Reikalingos, bet nepateikiamos medžiagos

• GeneXpert Dx sistema arba GeneXpert Infinity sistema (katalogo numeris skiriasi priklausomai nuo konfigūracijos): GeneXpert instrumentas, kompiuteris su patentuotos GeneXpert programinės įrangos versija 4.3 ar vėlesne, brūkšninių kodų skaitytuvas, naudojimosi vadovas.
• Spausdintuvas: jei reikia spausdintuvo, susisiekite su Cepheid pardavimo atstovu dėl rekomenduojamo spausdintuvo įsigijimo.
• Vortekso tipo purtyklė.
• Vienkartiniai sterilūs išpilstymo dozatoriai.
• Mėginių surinkimo priemonė, pavyzdžiui Cepheid mėginių paėmimo priemonė (Cepheid katalogo nr. 900-0370), Cepheid vienkartinis tamponas (Cepheid katalogo nr. SDPS-120), ar Copan dviguba paėmimo ir transportavimo sistema (139C LQ STUART).

7. Įspėjimai ir atsargumo priemonės

- Su visais biologiniais mėginiais, įskaitant panaudotas kasetes, elkitės kaip su galinčiais pernešti infekcinius agentus. Kadangi nėra žinoma, kuris mėginys yra infekciškas, su visais biologiniais mėginiais reikia dirbti laikantis universaliųjų atsargumo priemonių. Gairės dėl darbo su mėginiais yra pateikiamos JAV Ligų kontrolės ir prevencijos centro ir Klinikinių ir laboratorinių standartų instituto.[23,24]
- Dėl darbo su chemikalais ir biologiniais mėginiais, laikykitės Jūsų laboratorijoje atliekamų saugos procedūrų.
- Dėvėkite švarius laboratorinius chalatus ir pirštines. Po kiekvieno mėginio apdorojimo

pasikeiskite pirštines.
• Xpert *C. difficile* BT tyrimo reagentų negalima sukeisti su kitais reagentais.
• Neatidarykite Xpert *C. difficile* BT kasetės dangtelio, išskyrus tą momentą, kai dedate mėginį ir reagentus arba išimate mėginį iš originalios kasetės, jog atliktumėte pakartotinį tyrimą su nauja kasete.
• Nenaudokite kasetės, kuri buvo išmesta ar supurtyta po to, kai įdėjote mėginį ir reagentą.
• Nepurtykite kasetės. Kasetės purtymas ar išmetimas ant žemės po atidarymo gali sukelti klaidingus rezultatus.
• Xpert *C. difficile* tyrimas neteikia jautrumo rezultatų. Jautrumo tyrimo atlikimui auginant kultūras yra reikalingas papildomas laikas.
• Nenaudokite kasetės, kurios reakcijos mėgintuvėlis yra pažeistas.
• Neklijuokite mėginio ID etiketės ant kasetės dangtelio ar ant brūkšnio kodo.
• Kiekviena vienkartinio naudojimo Xpert *C. difficile* BT kasetė yra skirta vieno tyrimo atlikimui. Kasečių nenaudokite pakartotinai.
• Dėl tinkamo kasečių ir nepanaudotų reagentų išmetimo pasitarkite su savo įstaigos atliekų utilizavimo skyriumi. Ši medžiaga gali turėti valstybinio pavojingų atliekų charakteristiką, kurioms yra taikomi specifiniai utilizavimo reikalavimai. Įstaigos turi laikytis savo šalyje galiojančių nurodymų dėl pavojingų atliekų utilizavimo.
• Darbo vietos ar įrangos užteršimo mėginiais ar kontrolėmis atveju užterštą vietą kruopščiai išvalykite chloro baliklio tirpalu, skiestu santykiu 1:10 ir užterštą paviršių nuvalykite 70% etanoliu. Prieš tęsiant darbą, paviršių gerai nusausinkite.

8. Cheminis pavojus[25,26]

8.1 Reagentas 2:

• Sudėtyje yra natrio hidroksido
• Signalinis žodis: įspėjimas
• CLP/GHS pavojaus frazės: H302: pavojingas prarijus, H315: dirgina odą, H319: sukelia rimtą akių sudirgimą.

8.2 Mėginio reagentas:

• Sudėtyje yra guanidinio tiocianato
• Signalinis žodis: įspėjimas
• CLP/GHS pavojaus frazės: H302: pavojingas prarijus, H412: pavojingas vandens organizmams su ilgai išliekančiu poveikiu, EUH031: sąlytyje su rūgštimis skleidžia toksiškas dujas.
• Atsargumo frazės:
• P264: Po darbo kruopščiai nusiplaukite rankas.
• P280: Dėvėkite apsaugines pirštines/akių apsaugą/veido apsaugą.
• P273: Venkite produkto patekimo į aplinką.
• P302 + P352: ANT ODOS: gausiai plaukite muilu ir vandeniu.
• P305 + P351 + P338: Į AKIS: keletą minučių kruopščiai plaukite vandeniu. Išimkite kontaktinius lęšius jei jie yra ir jei tai įmanoma padaryti. Plaukite toliau.
• P312: Jei pasijutote blogai, skambinkite APSINUODIJIMŲ CENTRUI ar gydytojui.
• P501: Turinį/konteinerį išmeskite laikydamiesi vietinių ir regioninių/valstybinių/tarptautinių taisyklių.
• P362: Nusivilkite užterštus rūbus ir prieš pakartotinį dėvėjimą juos išskalbkite.
• P321: Specifinis gydymas, dėl pirmosios pagalbos priemonių skaitykite papildomą informaciją.
• P332 + P313: Atsiradus odos sudirgimui, kreipkitės į gydytoją.
• P337 + P313: Jei akių sudirgimas išlieka, kreipkitės į gydytoją.

9. Mėginio surinkimas ir transportavimas

1. Beformes išmatas surinkite į sterilų konteinerį. Laikykitės Jūsų įstaigoje taikomų instrukcijų dėl mėginių, skirtų *C. difficile* BT tyrimui, surinkimo.
2. Konteinerį pažymėkite mėginio ID ir siųskite į laboratoriją ištyrimui.
3. Mėginius laikykite prie 2−8 °C. Mėginiai yra stabilus iki 5 dienų, jei yra laikomi prie 2−8 °C. Kambario temperatūroje (20−30 °C) mėginiai gali būti laikomi iki 24 valandų.

10. Procedūra

Kasetės paruošimas

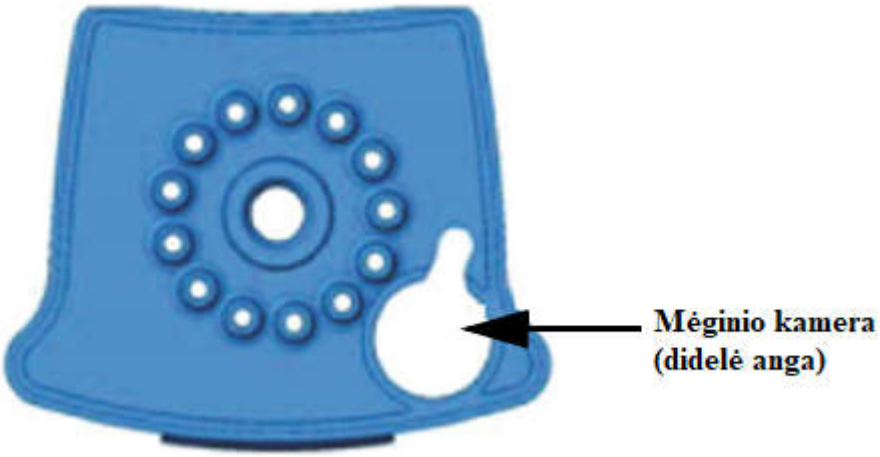
Svarbu: **tyrimą būtina pradėti per 30 minučių nuo reagentų įdėjimo į kasetę momento**.

Mėginio ir reagentų įdėjimas į kasetę (*Xpert C. Difficile* BT):

1. Iš pakuotės išimkite kasetę ir reagentus.
2. Trumpam pamerkite tamponėlį į beformių išmatų mėginį. Nereikia, kad tamponėlis visiškai permirktų.
3. Tamponėlį įdėkite į mėgintuvėlį su mėginio reagentu.

Pastaba: norint išvengti užterštumo, naudokite sterilią marlę.

4. Tamponėlį laikydami už kotelio šalia mėgintuvėlio krašto, kelis milimetrus pakelkite nuo mėgintuvėlio dugno ir stumdami kotelį į mėgintuvėlio kraštą, jį nulaužkite. Įsitikinkite, jog tamponėlis netrukdys užsukti kamštelio.
5. Užkimškite ir vorteksuokite dideliu greičiu 10 sekundžių.
6. Atidarykite kasetės dangtelį. Naudodamiesi švariu dozatoriumi, perneškite visą mėginio reagento turinį į Xpert *C. difficile* BT kasetės mėginio kamerą. Žr. 1 pav.
7. Uždarykite kasetės dangtelį.



Pav. 1. Xpert *C. difficile* BT tyrimo kasetė (vaizdas iš viršaus).

10.2 Tyrimo paleidimas

Svarbu: **prieš pradedant tyrimą, įsitikinkite, jog Xpert *C. difficile* BT tyrimo aprašymo byla yra importuota į programinę įrangą. Šiame skyriuje yra pateikiami pagrindiniai tyrimo atlikimo etapai. Dėl detalesnių instrukcijų prašome žiūrėti *GeneXpert Dx* sistemos naudojimosi vadovą ar *GeneXpert Infinity* sistemos naudojimosi vadovą**.

Pastaba: atliekami veiksmai gali skirtis, jei administratorius pakeitė pirminius sistemos darbo eigos nustatymus.

1. Įjunkite GeneXpert Dx instrumento sistemą:

• Jei naudojate GeneXpert Dx instrumentą, pirmiausia įjunkite instrumentą ir tik tada - kompiuterį. GeneXpert programinė įranga įsijungs automatiškai arba reikės dukart paspausti GeneXpert Dx programinės įrangos piktogramą, esančią Windows® darbalaukyje.

arba
• Jei naudojate GeneXpert Infinity instrumentą, įjunkite instrumentą. GeneXpert programinė įranga įsijungs automatiškai arba reikės dukart paspausti Xpertise programinės įrangos piktogramą, esančią Windows darbalaukyje.
2. Prisijunkite prie GeneXpert Dx instrumento sistemos programinės įrangos įvesdami savo vartotojo vardą ir slaptažodį.
3. GeneXpert Dx sistemos lange paspauskite elementą **Create Test** (GeneXpert Dx) (sukurti tyrimą) arba spustelėkite **Orders** (užsakymai) ir **Order Test** (užsakyti tyrimą) (Infinity). Atsidarys tyrimo sukūrimo langas.
4. Nuskenuokite paciento ID (pasirinktinai). Jei paciento ID įvedate rankiniu būdu, įsitikinkite, jog paciento ID įvedėte teisingai. Paciento ID yra susiejamas su tyrimo rezultatais, rodomais rezultatų peržiūros lange.
5. Sample ID (mėginio ID) lentelėje nuskenuokite arba įveskite mėginio ID. Įsitikinkite, kad paciento ID įvedėte teisingai. Mėginio ID bus susietas su tyrimo rezultatais ir bus rodomas "View Results" (peržiūrėti rezultatus) lange.
6. Nuskenuokite Xpert *C. difficile* BT kasetės brūkšninį kodą. Naudojantis brūkšninio kodo informacija, programinė įranga užpildys šių laukelių informaciją: „Select Assay" (pasirinkti tyrimą), „Reagent Lot ID" (reagento serijos ID), „Cartridge SN" (kasetės SN) ir „Expiration Date" (galiojimo data).
Pastaba: jei Xpert *C. difficile* BT kasetės brūkšninis kodas nenusiskenuoja, tyrimą pakartokite naudodami naują kasetę ir laikydamiesi procedūros, pateikiamos 15 skyriuje „Tyrimo pakartojimo procedūra".
7. Paspauskite **Start Test** (pradėti tyrimą) (GeneXpert Dx) arba **Submit** (pateikti) (Infinity). Atsiradusioje lentelėje įveskite savo slaptažodį.
8. Jei naudojate GeneXpert Infinity sistemą, padėkite kasetę ant konvejerio juostos. Kasetė bus įkelta automatiškai, tyrimas bus paleistas, o panaudota kasetė atsidurs atliekų konteineryje.
arba
Jei naudojate GeneXpert Dx instrumentą:
A. Atidarykite instrumento modulio dureles su žybsinčia žalia lempute ir įdėkite kasetę.
B. Uždarykite dureles. Tyrimas prasidės, o žalia leputė nustos blyksėti. Pasibaigus tyrimui, lemputė užges.
C. Palaukite, kol sistema atrakins dureles, atidarykite jas ir išimkite kasetę.
D. Panaudotas kasetes išmeskite į atitinkamą mėginių konteinerį laikydamiesi Jūsų įstaigoje praktikuojamų standartų.

11. Rezultatų peržiūra ir spausdinimas

Šiame skyriuje yra pateikiami pagrindiniai peržiūros ir spausdinimo etapų veiksmai. Dėl išsamių rezultatų peržiūros ir spausdinimo instrukcijų, žiūrėkite *GeneXpert Dx sistemos naudotojo vadovą* arba the *GeneXpert Infinity sistemos naudotojo vadovą*.
1. Paspauskite piktogramą **View Results** (peržiūrėti rezultatus).
2. Tyrimui pasibaigus, paspauskite klavišą **Report** (ataskaita), esantį rezultatų peržiūros lange ir peržiūrėkite rezultatus arba sugeneruokite ataskaitos PDF failą.

12. Kokybės kontrolė

Kiekviename tyrime yra mėginio apdorojimo kontrolė (SPC) ir mėgintuvėlio patikrinimo kontrolė (PCC).
Mėginio apdorojimo kontrolė (SPC)—užtikrina teisingą mėginio apdorojimą. SPC sudėtyje yra *Bacillus globigii* sporų sausų sporų rutuliuko formoje. Ši kontrolė yra kiekvienoje kasetėje tam, kad užtikrinti adekvatų mėginio bakterijos apdorojimą. SPC patvirtina, kad, esant organizmams, įvyko *C. difficile* bakterijos lizė ir mėginio apdorojimas yra adekvatus. Be to, ši kontrolė aptinka su

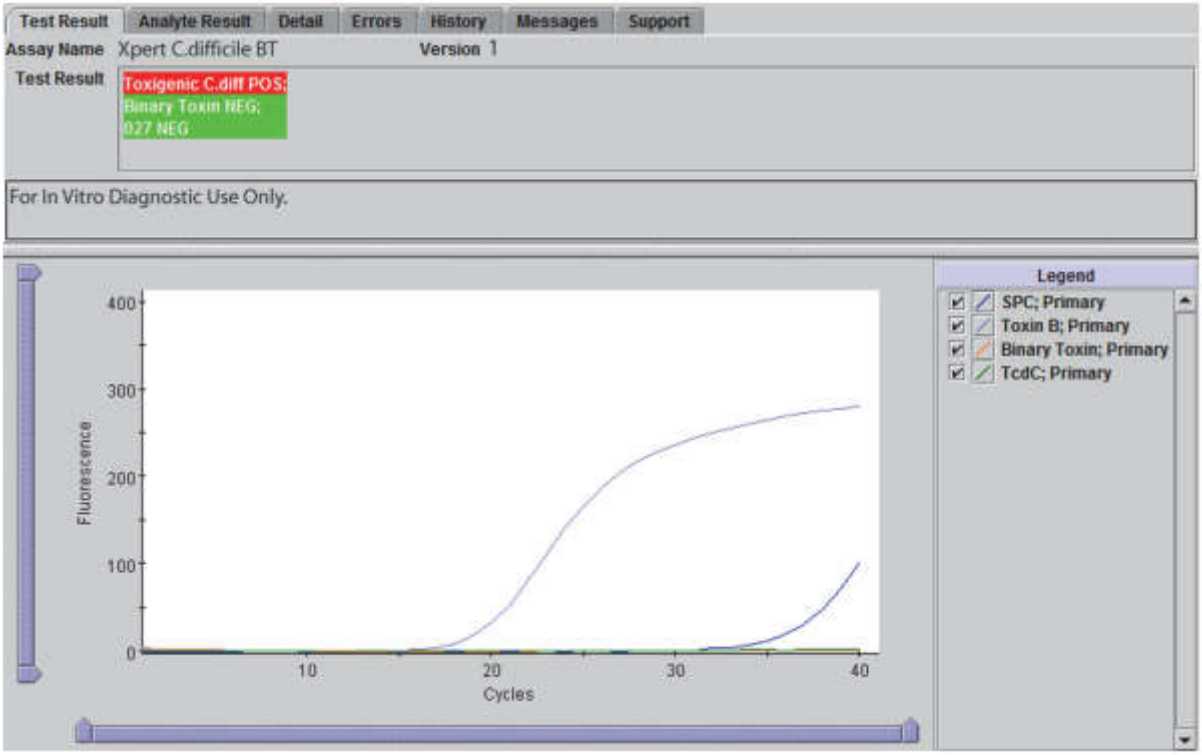
mėginiu susijusį tikro laiko PGR tyrimo inhibavimą, patikrina, ar PGR reakcijos sąlygos (temperatūra ir laikas) yra tinkamos amplifikacijos reakcijai ir, ar PGR reagentai yra funkcionalūs. SPC turi būti teigiama neigiamame mėginyje ir gali būti neigiama arba teigiama teigiamame mėginyje. SPC pavyksta, jei atitinka patvirtintus priimtinumo kriterijus.
Mėgintuvėlio patikrinimo kontrolė (PCC)—prieš pradedant PGR reakciją, GeneXpert sistema matuoja fluorescencijos signalą iš mėgintuvėlių ir patikrina rutuliukų rehidraciją, reakcijos mėgintuvėlio užpildymą, mėgintuvėlio integralumą ir dažų stabilumą. Mėgintuvėlio patikra pavyksta, jei atitinka patvirtintus priimtinumo kriterijus.

13. Rezultatų interpretavimas
GeneXpert instrumentų sistemos rezultatus interpretuoja pagal išmatuotą fluorescencijos signalą, įtraukiant apskaičiavimo algoritmus. Rezultatai yra rodomi "View Results" (Rezultatų peržiūros) lange. Galimi rezultatai yra pateikiami 1 lentelėje.

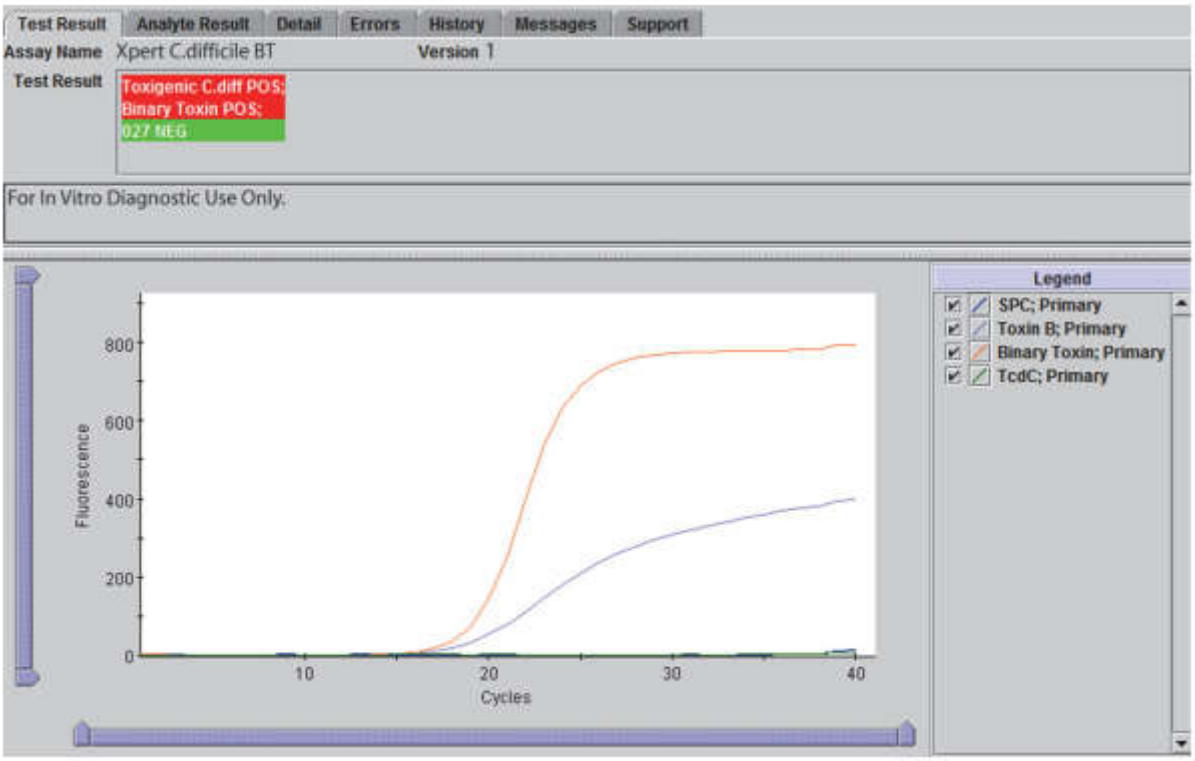
Rezultatas	**Interpretavimas**
Toxigenic C. diff POS, Binary Toxin NEG, 027 NEG Žr.pav. 2.	Aptiktos toksino, produkuojančio *C. difficile* taikinio DNR sekos. • Toksino, produkuojančio *C. difficile* — toksino, produkuojančio *C. difficile* (toksino B genas) taikinys turi teisingas Ct ribas ir galutinis taškas yra virš minimalaus nustatymo. • Binarinio toksino genas ir tcdC delecija ties nt 117 neaptikti. • SPC — NA (netaikoma); SPC yra ignoruojama, nes *C. difficile* amplifikacija gali konkuruoti su šia kontrole. • Probe Check — PASS (pavyko); mėgintuvėlio patikrinimas atliktas sėkmingai.
Toxigenic C. diff POS, Binary Toxin POS, 027 NEG Žr.pav. 3.	Aptiktos toksino, produkuojančio *C. difficile* taikinio DNR sekos. • Toksino, produkuojančio *C. difficile* taikinių (toksino B genas plius binarinio toksino genas) Cts ribos yra tinkamose ribose, o galutinis taškas yra virš minimalaus nustatymo; tcdC delecija ties nt 117 neaptikta. • SPC — NA (netaikoma); SPC yra ignoruojama, nes *C. difficile* amplifikacija gali konkuruoti su šia kontrole. • Probe Check — PASS (pavyko); mėgintuvėlio patikrinimas atliktas sėkmingai.
Toxigenic C. diff POS, Binary Toxin POS, 027 PRESUMPTIVE POS Žr.pav. 4	Aptiktos toksino, produkuojančio *C. difficile* ir numanomo 027 taikinio DNR sekos. • Visi toksinai, produkuojantys *C. difficile*, numanomi 027 taikiniai (toksinas B, binarinis toksinas ir tcdC delecija ties nt 117) turi teisingas Ct ribas ir galutinis taškas yra virš minimalaus nustatymo. • SPC — NA (netaikoma); SPC yra ignoruojama, nes *C. difficile* amplifikacija gali konkuruoti su šia kontrole. • Probe Check — PASS (pavyko); mėgintuvėlio patikrinimas atliktas sėkmingai.
Toxigenic C. diff NEG, Binary Toxin POS, 027 NEG Žr.pav.5	*C. difficile* toksino B sekos nėra aptiktos; tačiau aptiktas kitas DNR taikinys (binarinio toksino genas), kurio Cts ribos yra tinkamose ribose, o galutinis taškas yra virš minimalaus nustatymo. Teigiamo binarinio toksino klinikinė reikšmė dar turi būti nustatoma. • SPC — NA (netaikoma); SPC yra ignoruojama, nes *C. difficile* amplifikacija gali konkuruoti su šia kontrole. • Probe Check — PASS (pavyko); mėgintuvėlio patikrinimas atliktas sėkmingai.

Toxigenic C. diff NEG, Binary Toxin NEG, 027 NEG Žr.pav.6.	*C. difficile* taikinio DNR sekos (toksino B genas, binarinio toksino genas) neaptiktos. • Toksino, produkuojančio *C. difficile* taikinių (toksino B genas ir binarinio toksino genas) neaptikta; kiti DNR taikiniai dėl toksigeninio *C.difficile* (tcdC delecija ties nt 117) neaptikti. • SPC — PASS (pavyko); SPC Ct yra tinkamose ribose, o galutinis taškas yra virš minimalaus nustatymo. • Probe Check — PASS (pavyko); mėgintuvėlio patikrinimas atliktas sėkmingai.
INVALID Žr.pav.7	*C. difficile* taikinio DNR buvimas ar nebuvimas negali būti nustatytas; pakartokite tyrimą pagal 15 skyriaus instrukcijas. SPC neatitinka priimtinumo kriterijų, mėginys nebuvo tinkamai apdorotas arba PGR yra inhibuota. • INVALID — *C. difficile* taikinio DNR buvimas ar nebuvimas negali būti nustatytas. • SPC — FAIL (nepavyko); SPC taikinio rezultatas yra neigiamas ir SPC Ct nepatenka yra teisingas ribas, o galutinis taškas yra žemiau minimalaus nustatymo. • Probe Check — PASS (pavyko); mėgintuvėlio patikrinimas atliktas sėkmingai.
ERROR	*C. difficile* taikinio DNR buvimas ar nebuvimas negali būti nustatytas; pakartokite tyrimą pagal 15 skyriaus instrukcijas. Mėgintuvėlio patikrinimas nėra sėkmingas dėl netinkamai užpildyto mėgintuvėlio, mėgintuvėlio integralumo problemos ar dėl viršytų slėgio ribų. • Toxin B (toksinas B)— NO RESULT (NĖRA REZULTATO) • Binary Toxin (binarinis toksinas) — NO RESULT (NĖRA REZULTATO) • tcdC deletion at nt 117 (tcdC delecija ties nt 117) — NO RESULT (NĖRA REZULTATO) • *SPC — NO RESULT (NĖRA REZULTATO) • Probe Check (mėgintuvėlio patikrinimas) — FAIL* (nepavyko); vieno ar kelių mėgintuvėlių patikrinimas nėra sėkmingas. * Jei mėgintuvėlio patikrinimas yra sėkmingas, klaida įvyko dėl sistemos komponento.
NO RESULT	*C. difficile* taikinio DNR buvimas ar nebuvimas negali būti nustatytas; pakartokite tyrimą pagal 15 skyriaus instrukcijas. Surinkta nepakankamai duomenų tyrimo rezultatų gavimui (pvz., operatorius sustabdė tyrimą tyrimo eigos metu). • Toxin B (toksinas B) (tcdB)— NO RESULT (NĖRA REZULTATO) • Binary Toxin (binarinis toksinas) (cdt) — NO RESULT (NĖRA REZULTATO) • tcdCΔ117 — NO RESULT (NĖRA REZULTATO) • SPC — NO RESULT (NĖRA REZULTATO) • Probe Check (mėgintuvėlio patikrinimas) — NA (netaikoma)

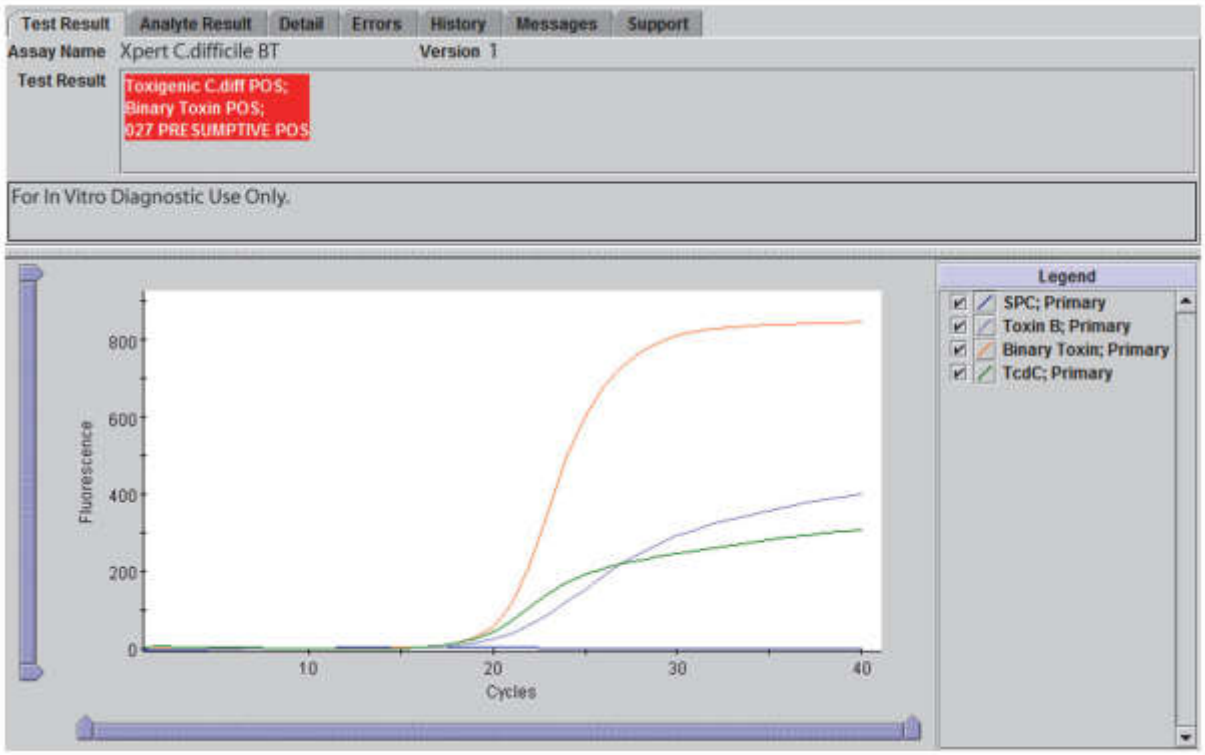
Pastaba: ekranų paveikslėliai, pateikiami šiame skyriuje (pav. 2, pav. 3, pav. 4, pav. 5, pav. 6 ir pav. 7) yra iš GeneXpert Dx instrumento, veikiančio su GeneXpert Dx programine įranga.



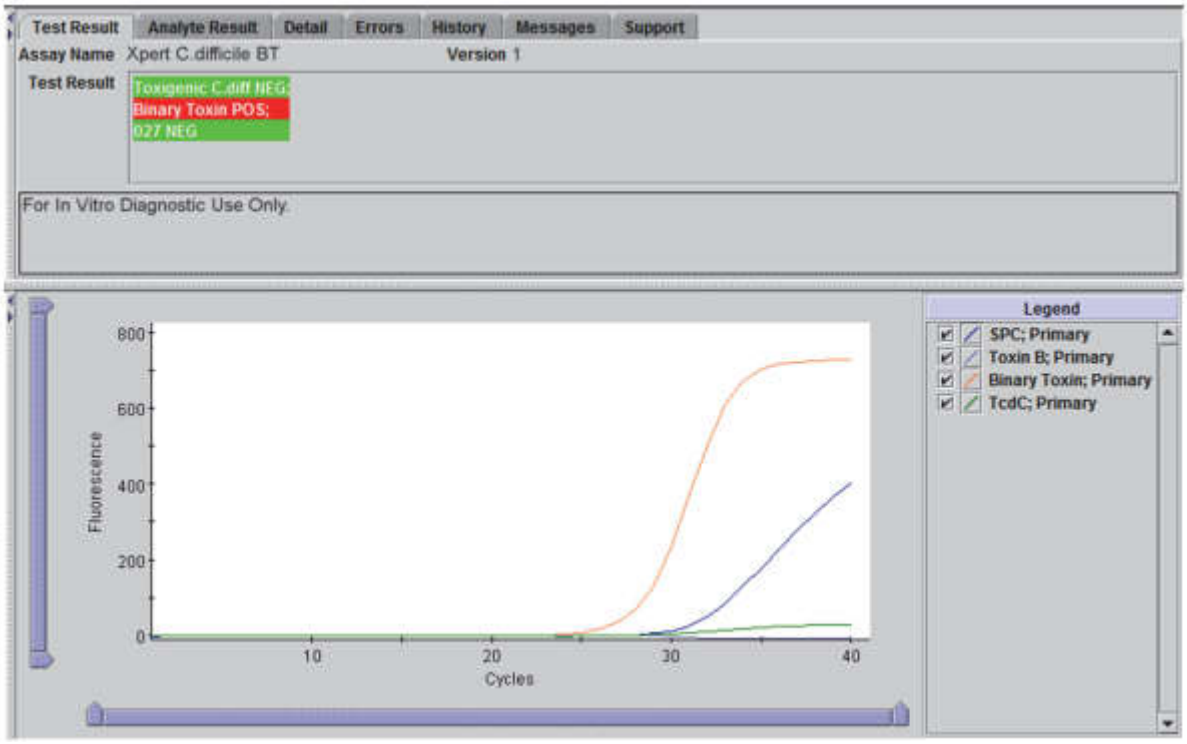
Pav. 2. Teigiamo toksigeninio C. Diff, neigiamo binarinio toksino ir neigiamo 027 rezultato pavyzdys



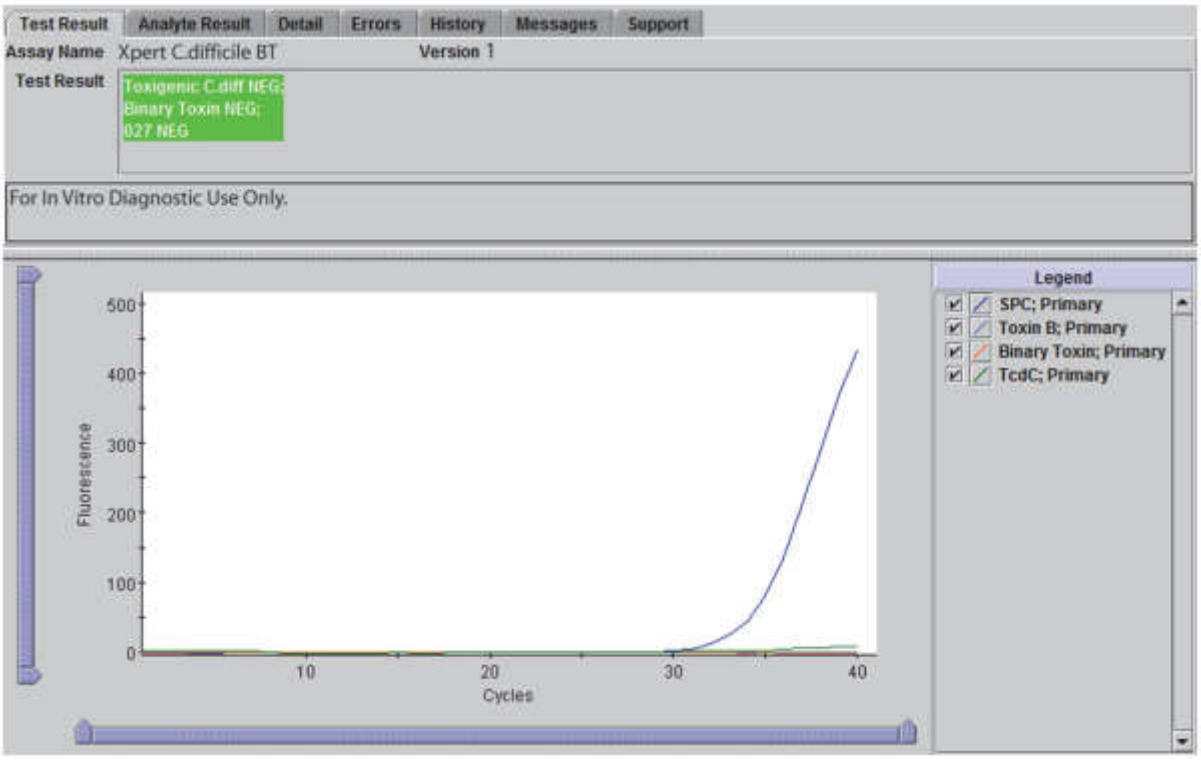
Pav. 3. Teigiamo toksigeninio C. Diff, teigiamo binarinio toksino ir neigiamo 027 rezultato pavyzdys



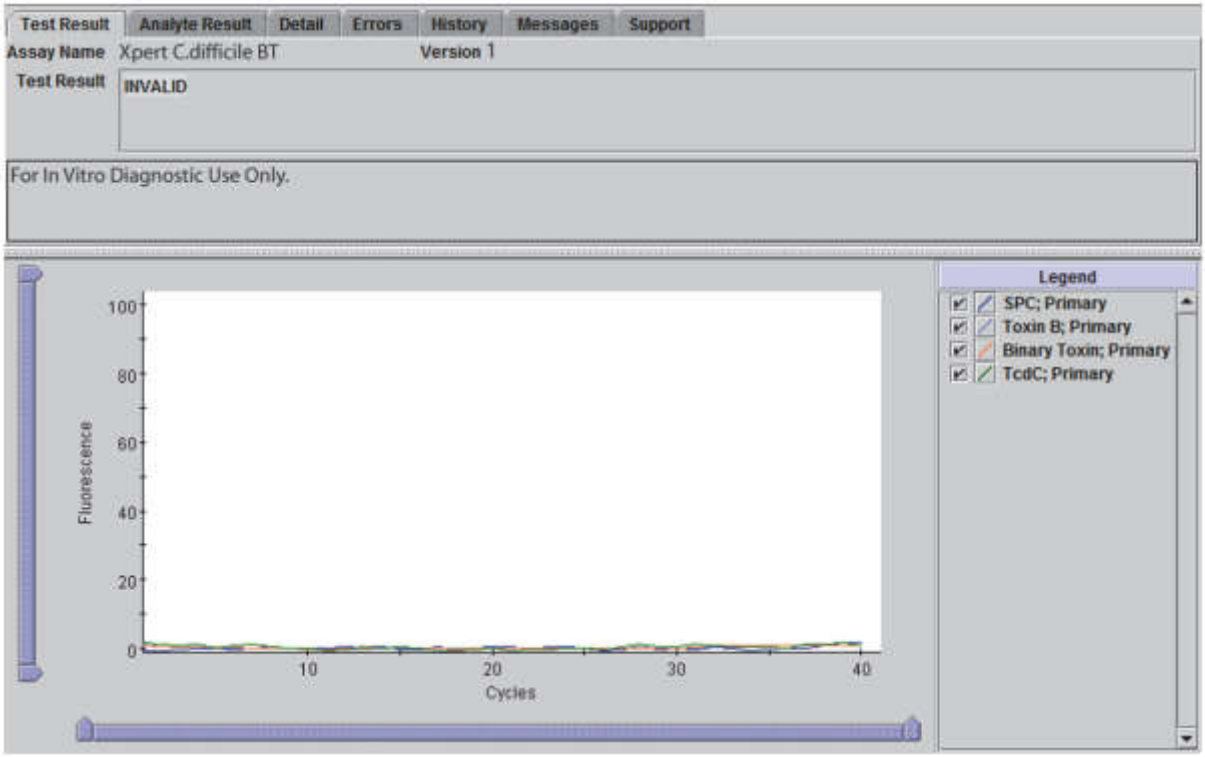
Pav. 4. Teigiamo toksigeninio C. diff, teigiamo binarinio toksino ir numanomai teigiamo 027 rezultato pavyzdys



Pav. 5. Neigiamo toksigeninio C. diff, teigiamo binarinio toksino ir neigiamo 027 rezultato pavyzdys



Pav. 6. Neigiamo toksigeninio C. diff, neigiamo binarinio toksino ir neigiamo 027 rezultato pavyzdys



Pav. 7. Klaidingo rezultato pavyzdys

14. Tyrimo kartojimo priežastys

Tyrimą kartokite laikydamiesi 15 skyriuje pateiktų instrukcijų, jei gavote vieną šių tyrimų rezultatų:
• **INVALID** (KLAIDINGAS) rezultatas rodo, kad SPC nepavyko. Mėginys nebuvo tinkamai paruoštas arba PGR buvo inhibuotas.

• **ERROR** (KLAIDA) rezultatas rodo, kad mėgintuvėlio patikrinimo kontrolė buvo nesėkminga, o tyrimas buvo atšauktas galimai dėl to, kad reakcijos mėgintuvėlis nebuvo tinkamai užpildytas, buvo aptikta reagento mėgintuvėlio integralumo problema, buvo peržengtos maksimalios slėgio ribos arba buvo aptikta vožtuvo pozicijos klaida.
• **NO RESULT** (NĖRA REZULTATO) rodo, kad nėra pakankamai duomenų. Pavyzdžiui, operatorius sustabdė tyrimą tyrimo metu.

15. Pakartotinio tyrimo procedūra
Norint pakartotinai ištirti neaiškaus rezultato mėginį per 3 valandas: naudokite naują kasetę (pakartotinai naudoti kasetės negalima) ir naujus reagentus.
1. Iš rinkinio pakuotės išimkite naują kasetę.
2. Naudodami vienkartinį dozatorių, perneškite visą likusį turinį iš mėginio kameros į naują mėginio reagento buteliuką.
3. Vorteksuokite ir visą mėginio reagento turinį perneškite į naujos Xpert *C. difficile* BT kasetės mėginio kamerą.
4. Uždarykite dangtelį ir pradėkite naują tyrimą.
Norint pakartotinai ištirti neaiškaus rezultato mėginį po 3 valandų, tyrimą kartokite naudodami naują mėginį, paimtą iš originalaus paciento mėginio.

16. Apribojimai
• Ne 027 izoliatai, atstovaujantys toksinotipą XIV, bus pateikiami kaip **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS,** naudojant Xpert *C. difficile* BT tyrimą.
• **Toxigenic C. diff NEG; Binary Toxin POS, Presumptive 027 NEG** su Xpert *C. difficile* BT gali slėpti toksino B geną ir/ar *tcdC* deleciją, jei jis bus žemiau tyrimo aptikimo ribos.
• Kartais, ne 027 izoliatai, atstovaujantys toksinotipus IV, V ir X, gali būti pateikiami kaip **Toxigenic C. diff POS; Binary Toxin POS; 027 PRESUMPTIVE POS,** naudojant Xpert *C. difficile* BT tyrimą.
• Xpert *C. difficile* BT tyrimo veiksmingumas patvirtintas naudojant procedūras, pateiktas šiame pakuotės aprašyme. Šių procedūrų modifikacijos gali paveikti tyrimo veiksmingumą.
• Xpert *C. difficile* BT tyrimo rezultatai turi būti interpretuojami kartu su kitais laboratoriniais ir klinikiniais duomenimis, prieinamais gydytojui.
• Klaidingi rezultatai gali atsirasti dėl netinkamo mėginio surinkimo, mėginio surinkimo procedūros nesilaikymo, netinkamo mėginio paruošimo ar laikymo, technikinių klaidų, mėginių sumaišymo arba dėl per mažo organizmų skaičiaus mėginyje. Norint išvengti klaidingų rezultatų, būtina laikytis šiame pakuotės aprašyme pateiktų instrukcijų.
• Dėl skiedimo faktoriaus pakartotinio tyrimo procedūroje, įmanoma, jog *C. difficile* teigiamo mėginiai, esantys šalia ar ties Xpert *C. difficile* BT tyrimo aptikimo riba, pakartotiniame tyrime demonstruos klaidingai neigiamą rezultatą.
• Xpert *C. difficile* BT tyrimo inhibicija buvo pastebėta esant šioms substancijoms: cinko oksido pasta ir Vagisil® kremas.
• CDI protrūkius gali sukelti kitos nei 027 padermės.
• Klaidingai neigiami rezultatai gali atsirasti, kai infekuotas organizmas turi genominių mutacijų, insercijų, delecijų ar pertvarkymų, įvykusių ankstyvojoje susirgimo stadijoje.
• Teigiami rezultatai, gauti iš imunosupresuotų pacientų, gali atspindėti asimptomatinį *C. difficile* pernešimą.
• *C. difficile* nukleininių rūgščių aptikimas išmatose patvirtina organizmų buvimą pacientuose su diarėja, bet neindikuoja, jog *C. difficile* yra diarėjos priežastis.
• Veiksmingumo charakteristika nėra nustatyta pacientams, jaunesniems nei 2 metų amžiaus.

17. Tikėtinos vertės

Xpert *C. difficile* BT tyrimo klinikinėje studijoje iš viso buvo naudota 2293 beformių išmatų mėginiai, surinkti iš septynių centrų JAV ir Kanadoje. Teigiamo toksigeninio *C. difficile* skaičius ir procentinė išraiška, apskaičiuota pagal amžių ir lytį, yra pateikiama 2 lentelėje ir 3 lentelėje.

2 lentelė. Toksigeninio *C. difficile* paplitimas pagal amžiaus grupę

Amžiaus grupė	N	Toksigeninio C.difficile paplitimas (įskaitant 027)	Binarinio toksino paplitimas	027 paplitimas
2-5	16	37.5% (6/16)	12.5% (2/16)	12.5% (2/16)
6-21	105	12.4% (13/105)	2.9% (3/105)	0.9% (1/105)
22-59	898	16.4% (147/898)	4.8% (43/898)	3.3% (30/898)
>60	1274	20.7% (264/1274)	9.2% (117/1274)	7.2% (92/1274)
Iš viso	2293	18.8% (430/2293)	7.2% (165/2293)	5.5% (125/2293)

a. Paplitimas paremtas Xpert rezultatais.

3 lentelė. Toksigeninio *C. difficile* paplitimas pagal lytį

Lytis	N	Toksigeninio C.difficile paplitimas (įskaitant 027)	Binarinio toksino paplitimas	027 paplitimas
Vyrai	1072	18.2% (195/1072)	6.3% (68/1072)	5.0% (54/1072)
Moterys	1221	19.2% (235/1221)	7.9% (97/1221)	5.8% (71/1221)
Iš viso	2293	18.8% (430/2293)	7.2% (165/2293)	5.5% (125/2293)

18. Veiksmingumo charakteristika

18.1 Klinikinis veiksmingumas

Xpert *C. difficile* BT veiksmingumo charakteristika buvo nustatoma atliekant prospektinę tiriamąją studiją septyniose JAV ir Kanados institucijose, Xpert *C. difficile* BT tyrimą lyginant su referentiniu kultivavimo metodu kartu su CCCN tyrimu su izoliatų ir padermių tipavimu dėl toksigeninių padermių, atliekant PGR ribotipavimą.

Dalyvavo subjetai, kuriems buvo būtina atlikti *C. difficile* tyrimą. Buvo tiriama beformių išmatų dalis su Xpert *C. difficile* BT tyrimu. Likę mėginiai buvo siunčiami į centrinę laboratoriją referentiniam ištyrimui kultūros metodu ir citotoksino B tyrimui. Kiekvienas išmatų mėginys buvo inokuliuojamas ant preredukuoto cikloserino cefoksitino-fruktozės agaro – tiesiogiai į lėkštelę (CCFA-D) ir cikloserino cefoksitino manitolio buljono su taurocholato lizozimo cisteinu (CCMB-TAL). Po 24 valandų CCMB-TAL buvo subkultivuotas ant antros CCFA-E lėkštelės (CCFA-pagausinta). Šis tiesioginio pagausinimo kultivavimo metodas yra laikomas „referentiniu kultūros metodu“.

Jei *C. difficile* buvo izoliuota iš CCFA-D lėkštelės ir izoliatas buvo teigiamas su CCCN tyrimu, mėginys buvo klasifikuojamas kaip “teigiamas toksigeniniam *C. difficile* ” ir CCFA-E lėkštelė toliau nebuvo tiriama. Jei *C. difficile* nebuvo izoliuota iš CCFA-D lėkštelės ar jei izoliatas buvo neigiamas su CCCN tyrimu, CCFA-E lėkštelė buvo tiriama toliau.

Jei CCFA-E buvo teigiamas dėl *C. difficile* ir izoliatas buvo teigiamas su CCCN tyrimu, mėginys buvo klasifikuojamas kaip “ teigiamas toksigeniniam *C. difficile* ”. Mėginys buvo nustatytas kaip neigiamas, jei CCFA-E buvo neigiamas dėl *C. difficile* arba izoliatas buvo neigiamas su CCCN tyrimu.

Atlikus referentinį kultūros tyrimą, toksigeniniam *C. difficile* teigiami izoliatai buvo siunčiami į centrinę laboratoriją padermės identifikavimui atliekant PGR rivotipavimą.
Xpert *C. difficile* BT tyrimo veiksmingumas buvo apskaičiuotas atsižvelgiant į tiesioginio kultivavimo rezultatus su padermės tipavimu ir referentinį kultūros metodą su padermių tipavimu.

18.2 Suminiai rezultatai
Iš viso buvo tirti 2293 mėginiai su Xpert *C. difficile* BT tyrimu, kultūros metodu ir padermių tipavimu.

Veiksmingumo rezultatai ir tiesioginis kultivavimas
Lyginant su tiesioginio kultivavimo metodu ir PGR ribotipavimu, Xpert *C. difficile* BT tyrimas demonstravimo 98.78% jautrumą ir 90.86% specifiškumą toksigeniniam *C. difficile*. Xpert *C. difficile* BT tyrimas taip pat parodė 100% teigiamą atitikimą ir 97.70% neigiamą atitikimą dėl 027 (4 lentelė).

4 lentelė. Xpert *C. difficile* BT tyrimo veiksmingumas ir tiesioginis kultivavimas bei PGR ribotipavimas

Tiesioginis kultivavimas ir PGR ribotipavimas					
		Toksinas B+ 027+	**Toksinas B+ 027-**	**NEIG.**	**Iš viso[a]**
Xpert *C.difficile* BT[b]	**Toksinas B+ 027+**	74	4	47	125
	Toksinas B+ 027-	0	164	140	304
	NEIG.	0	3	1860	1863
	Iš viso	74	171	2047	2292
		Toksigeninis *C.difficile*		**Toksigeninis *C.difficile*/027**	
		Jautrumas: 98.78% (242/245) Specifiškumas:90.86% (1860/2047) Tikslumas:91.71% (2102/2292) PPV[c]: 56.41% (242/429) NPV[d]:99.84% (1860/1863)		Teig.atitikimas: 100% (74/74) Neig.atitikimas: 97.70% (2167/2218) Tikslumas: 97.77% (2241/2292) PPV: 59.20% (74/125) NPV: 100% (2218/2218)	

a. Vienas izoliatas buvo netipuojamas dėl užterštumo: šis mėginys nįra įtrauktas į veiksmingumo statistiką.
b. Rodomi pirmojo arba antrojo bandymo su Xpert rezultatai. Apie 3.2% mėginių buvo nenustatyti pirmojo bandymo metu.
c. Teigiama tikėtina vertė
d. Neigiama tikėtina vertė

Veiksmingumas ir referentinis kultivavimas
Lyginant su referentiniu kultivavimo metodu ir PGR ribotipavimu, Xpert *C. difficile* BT tyrimas demonstravo 93.39% jautrumą ir 94.02% specifiškumą toksigeniniam *C. difficile*. Xpert *C. difficile* BT tyrimas taip pat parodė 98.89% teigiamą atitikimą ir 98.36% neigiamą atitikimą dėl 027 (5 lentelė).

5 lentelė. Xpert *C. difficile* BT tyrimo veiksmingumas ir referentinis kultivavimas bei PGR ribotipavimas

Referentinis kultivavimas ir PGR ribotipavimas					
		Toksinas B+ 027+	**Toksinas B+ 027-**	**NEIG.**	**Iš viso[a]**
Xpert *C.difficile* BT[b]	**Toksinas B+ 027+**	89	5	31	125
	Toksinas B+ 027-	0	217	86	303
	NEIG.	1	21	1841	1863
	Iš viso	90	243	1958	2291
		Toksigeninis *C.difficile*		**Toksigeninis *C.difficile*/027**	
		Jautrumas: 93.39% (311/333) Specifiškumas:94.02% (1841/1958) Tikslumas:93.93% (2152/2291) PPV[c]: 72.66% (311/428) NPV[d]:98.82% (1841/1863)		Teig.atitikimas:98.89% (89/90) Neig.atitikimas: 98.36% (2165/2201) Tikslumas: 98.38% (2254/2291) PPV: 71.20% (89/125) NPV: 99.95% (2165/2166)	

a. Vienas izoliatas buvo netipuojamas dėl užterštumo: šis mėginys nįra įtrauktas į veiksmingumo statistiką.
b. Rodomi pirmojo arba antrojo bandymo su Xpert rezultatai. Apie 3.2% mėginių buvo nenustatyti pirmojo bandymo metu.
c. Teigiama tikėtina vertė
d. Neigiama tikėtina vertė

Santrauka
6 lentelėje yra pateikiamas skaičius mėginių, kiekvienam skirtingam tyrimo rezultatui iš 2293 mėginių, naudotų veiksmingumo duomenų analizėje.

6 lentelė. Bendras Xpert *C. difficile* BT tyrimo veiksmingumas

Tyrimo rezultatas	**N**
Toxigeniniam C. diff TEIG; Binariniam toksinui NEIG; O27 NEIG	272
Toxigeniniam C. diff TEIG; Binariniam toksinui TEIG; O27 NEG	36
Toxigeniniam C. diff TEIG; Binariniam toksinui TEIG; O27 NUMANOMAI TEIG	122
Toxigeniniam C. diff NEIG; Binariniam toksinui TEIG; O27 NEIG	7[a]
Toxigeniniam C. diff NEIG; Binariniam toksinui NEIG; O27 NEIG	1856
Iš viso	**2293**

a. Papildomame tyrime, 4 iš 7 padermių maskavo toksino B geną.

Antibiotikų vartojimas
Tarp 2293 atvejų, įskaitant pagrindinį duomenų rinkinį, antibiotikų vartojimas per 2 mėnesius iki mėginio paėmimo, buvo pateiktas 1630 atvejų, o antibiotikų nevartojimas – 570 atvejų; 93 atvejais antibiotikų vartojimo statusas buvo nežinomas. Antibiotikų vartojimas nesukėlė statistiškai reikšmingų skirtumų tyrimo veiksmingumo nustatyme.

19. Analitinis veiksmingumas

19.1 Analitinis specifiškumas

Penkiasdešimt penkios (55) padermės buvo surinktos, nustatytos kiekybiškai ir tirtos su Xpert *C. difficile* BT tyrimu. Padermės buvo gautos iš *American Type Culture Collection (ATCC), Culture Collection University of Göteborg (CCUG), German Collection of Microorganisms and Cell Cultures (DSMZ), the Centers for Disease Control and Prevention (CDC), the Institute of Public Health, Maribor, Slovenia and Swedish Institute for Infectious Disease Control (SMI).*

Į visas tirtas bakterijų rūšis, buvo įtraukta dešimt (10) ne toksigeninių *C. difficile* pademrių ir vienuolika (11) ne *C. difficile Clostridium* rūšių. Tirti organizmai buvo identifikuoti kaip arba Gram teigiami (37), arba Gram neigiami (18). Vėliau organizmai buvo identifikuojami kaip aerobiniai (24), anaerobiniai (29) ar mikroaerobiniai (2).

Kiekviena padermė buvo tirta trigubu pakartojimu ties koncentracijomis nuo 1.1x108 iki 2.2x1010 CFU/tepinėlyje. Studijoje buvo naudojamos teigiama ir neigiama kontrolės. Studijos sąlygomis, visi izoliatai buvo nustatyti kaip **toksigeniniam C. diff NEIG.; binariniam toksinui NEIG.; 027 NEIG.** (7 lentelė). Analitinis specifiškumas buvo 100%.

Buvo tirtos papildomos ne *difficile Clostridium* rūšys, kad būtų pademonstruotas binarinio toksino tyrimo specifiškumas.

7 lentelė. Binarinio toksino geno specifiškumo studijos rezultatai

Gentis	Rūšis	Tirtas skaičius	Toksinas A/B	Binarinis toksinas
Clostridium	*aldenense*	2	neig.	neig.
Clostridium	*aminovalericum-like*	2	neig.	neig.
Clostridium	*baratii*	2	neig.	neig.
Clostridium	*bartletti*	1	neig.	neig.
Clostridium	*bifermentans*	2	neig.	neig.
Clostridium	*bolteae*	2	neig.	neig.
Clostridium	*butyricum*	2	neig.	neig.
Clostridium	*cadaveris*	2	neig.	neig.
Clostridium	*celerecrescens*	2	neig.	neig.
Clostridium	*citroniae*	2	neig.	neig.
Clostridium	*clostridioforme*	2	neig.	neig.
Clostridium	*cochlearium*	1	neig.	neig.
Clostridium	*colicanis*	2	neig.	neig.
Clostridium	*disporicum*	1	neig.	neig.
Clostridium	*fallax*	2	neig.	neig.
Clostridium	*glycolicum*	2	neig.	neig.
Clostridium	*hastiforme*	1	neig.	neig.
Clostridium	*hathewayi*	2	neig.	neig.
Clostridium	*hylemonae*	2	neig.	neig.
Clostridium	*innocuum*	2	neig.	neig.
Clostridium	*lactatifermentans*	2	neig.	neig.
Clostridium	*lavalense*	1	neig.	neig.
Clostridium	*limosum*	2	neig.	neig.
Clostridium	*mangenotii*	1	neig.	neig.
Clostridium	*mayombei-like*	1	neig.	neig.
Clostridium	*novyi*	2	neig.	neig.
Clostridium	*paraputrificum*	2	neig.	neig.
Clostridium	*perfringens*	2	neig.	neig.

7 lentelė. Binarinio toksino geno specifiškumo studijos rezultatai (tęsinys)

Gentis	Rūšis	Tirtas skaičius	Toksinas A/B	Binarinis toksinas
Clostridium	*perfringens Type E*	3	neig.	neig.
Clostridium	*ramosum*	2	neig.	neig.
Clostridium	*sardiniense*	1	neig.	neig.
Clostridium	*scindens*	2	neig.	neig.
Clostridium	*septicum*	2	neig.	neig.
Clostridium	*sordellii*	2	neig.	neig.
Clostridium	species	19	neig.	neig.
Clostridium	*spiroforme*	1	neig.	neig.
Clostridium	*sporogenes*	2	neig.	neig.
Clostridium	*subterminale* group	3	neig.	neig.
Clostridium	*symbiosum*	2	neig.	neig.
Clostridium	*tertium*	2	neig.	neig.
Clostridium	*tetani*	1	neig.	neig.
Clostridium	*xylano/aerotolerans*	1	neig.	neig.
Clostridium	*difficile* RT 027	5	+	+
Clostridium	*difficile* RT 078	2	+	+

Visi ne binarinį toksą turintys izoliatai buvo neigiami su Xpert *C. difficile* BT tyrimu.

19.2 Analitinis jautrumas

Studijų metu buvo nustatomas 95% pasikliovimo intervalas analitinei aptikimo ribai (LoD), naudojant *C. Difficile,* skiestą žmogaus kilmės fekalijų matrica, kuri gali būti aptinkama Xpert *C. difficile* BT tyrimo. Fekalijų matrica buvo sudaryta iš skystų žmogaus išmatų (*C. difficile* neigiamas mėginys pagal Xpert *C. difficile* BT tyrimą), skiestų su PBS su 15% glicerolio. LoD yra mažiausias kolonijas formuojančių vienetų (CFU) skaičius tepinėlyje, kuris gali būti atkuriamai atskiriamas iš neigiamų mėginių su 95% pasikliovimu.

20 replikų buvo vertinama su kiekviena *C. difficile* tirta koncentracija (CFU/tepinėlyje) 7 skirtingoms *C. difficile* padermėms, atstovaujančios ribotipus 0 (dvi padermės), III (dvi padermės), IV, V, ir VIII (po vieną kiekvienos padermės).

Numanomas ir pasikliovimo intervalas buvo nustatomi naudojant logistinę regresiją su duomenimis (replikų teigiamų rezultatų skaičius kiekviename lygyje) visame CFU tirtame spektre. Pasikliovimo intervalas buvo nustatomas naudojant maksimaliai panašius įvertinimus su logistinio modelio parametrais, naudojant plačią įvairių mėginių kovariacijos matricą. LoD taško įvertinimai ir 95% viršutinis ir apatinis pasikliovimo intervalai kiekvienam tirtam *C. difficile* toksinotipui yra pateikti 8 lentelėje.

8 lentelė. *C. difficile* analitinės LoD 95% pasikliovimo intervalai

Padermės ID	Toksinotipas	$LoD_{95\%}$ (CFU/tepinėlyje)	Apatinis 95% PI	Viršutinis 95% PI
VPI 10463 (CCUG19126)	0	255	190	632
90556-M6S (ATCC9689)	0	460	419	587
LUMC-1 (027)[a]	III	23	19	31
LUMC-5 (027)[a]	III	75	45	176
LUMC-7	V	45	34	104
LUMC-6	VIII	60	50	74
9101	XII	41	34	49

a. Pagal PGR ribotipavimą.

Šios studijos rezultatai rodo, jog Xpert *C. difficile* BT tyrimas pateikia teigiamą *C. difficile* rezultatą 95% išmatų mėginiams su 460 CFU/tepinėlyje ir 027 numanomą teigiamą rezultatą 95% atvejų tepinėliams su 75 CFU.
Be LoD nustatymo, aštuoniolika *C. difficile* padermių, atstovaujančių toksinotipus 0 plius 12 skirtingų toksinotipų, įskaitant keturis 027 toksinotipo III izoliatus, buvo tirtos naudojant Xpert *C. difficile* BT tyrimą. *C. difficile* padermės atstovavo daugumą *C. difficile* toksinotipų, aptinkamų praktikoje. Kamieninės kultūros buvo paruoštos suspenduojant bakterinį augimą agaro lėkštelėse PBS buferyje su 15% glicerolio. Kiekvienos kultūros koncentracija buvo nustatyta nuo 1.4 iki 5.9 McFarland vienetų. Visos padermės buvo skiestos serijiniu būdu iki maždaug 900 CFU/tepinėlyje ir tirtos trigubu pakartojimu.
Studijos sąlygomis, Xpert *C. difficile* BT tyrimas teisingai identifikavo visas 18 padermių kaip **toksigeniniam C. diff TEIG.** Į panelį įtraukti 8 toksinotipai buvo nustatyti kaip teigiami dėl binarinio toksino (CDT) produkavimo. Visi buvo nustatyti kaip CDT teigiami su Xpert *C. difficile* BT tyrimu. Visi keturi 027 izoliatai, reprezentuojantys toksinotipą III, buvo teisingai identifikuoti kaip **toksigeniniam C. diff TEIG.; binariniam toksinui TEIG.; 027 NUMANOMAI TEIG.** Septyni *C. difficile* izoliatai iš PGR ribotipo 033 ir trys papildomi *C. difficile* izoliatai iš susijusio PGR ribotipo, kuris buvo neigiamas dėl *tcdA* ir tcdB, bet produkavo binarinį toksiną (CDT)22, buvo tirti su Xpert *C. difficile* BT tyrimu. Visi 10 izoliatų demonstravo teigiamą rezultatą tik dėl binarinio toksino (9 lentelė), taip patvirtinant tyrimo gebėjimą aptikti izoliatus, kurie yra toksinas A-, toksinas B-, binarinis toksinas +).

9 lentelė. Organizmų, produkuojančių tik binarinį toksiną (toksinas A-, toksinas B-), tyrimas su Xpert *C. difficile* BT

Organizmas	Padermės ID	PGR ribotipas	Tyrimo rezultatas
C. difficile	CD12-066	033	Toksigeniniam C.diff NEIG; binariniam toksinui TEIG; 027 NEIG
C. difficile	CD12-203	033	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	CD13-022	033	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	06-08-02	033	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	06-20-01	033	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	NT077	033	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	AI-0016	238	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	WA-0012	239	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	ES-0145	288	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG
C. difficile	R-0010	033	Toksigeniniam C.diff NEG; binariniam toksinui TEIG; 027 NEIG

19.3 Interferuojančios medžiagos
Dvidešimt viena (21) biologinė ir cheminė substancija, kartais naudojama ar randama išmatų mėginiuose, buvo tiriama dėl interferencijos su Xpert *C. difficile* BT tyrimu. Potencialiai interferuojančios medžiagos yra, bet neapsiriboja: Vagisil kremas ir cinko oksido pasta (žr. 16 skyrių). 10 lentelėje 19 pateiktų substancijų neparodė jokios aptinkamos interferencijos su Xpert *C. difficile* BT tyrimu.

10 lentelė. Stirtos substancijos, neinterferuojančios tyrimo

Substancija	**Substancija**
Bendras kraujas Karolinska University Hospital	K-Y Jelly/Gelée® McNeil-PPC
Mucinas (kiaulės) Sigma	Vazelinas Unilever
Kaopectate® Chattem	Dulcolax® Boehringer Ingelheim Pharmaceuticals
Immodium® McNeil-PPC	Preparation H Portable Wipes Wyeth Consumer Healthcare
Pepto-Bismol® Proctor & Gamble	Vaginalinė kontraceptinė plėvelė (VCF) Apothecus Pharmaceutical
Preparation H® Wyeth Consumer Healthcare	Vankomicinas Fluka
Fleet® CB Fleet Company	Metronidazolas Actavis
Riebalai išmatose Karolinska University Hospital	Anusol® Plus TM Warner-Lambert Company
Monistat® McNeil-PPC	E-Z HDTM didelio tankio bario sulfatas suspensijai E-Z EM Canada
Hidrokortizono kremas Longs Drugs	

20. Atkuriamumas
7 mėginių panelis su skirtingomis toksigeninio *C. difficile* ir *C. difficile* ribotipo 027 koncentracijomis, buvo tiriamas 10 skirtingų dienų, dviejų skirtingų operatorių, trijose skirtingose vietose (7 mėginiai x 2 operatoriai/diena x 10 dienų x 3 vietos). Visose 3 tyrimo vietose buvo naudojama viena Xpert *C. difficile* BT tyrimo partija. Xpert *C. difficile* BT tyrimai buvo atliekami laikantis Xpert *C. difficile* BT tyrimo procedūros. Rezultatai yra pateikti 11 ir 12 lentelėse.

11 lentelė. Atkuriamumo rezultatų (visų) santrauka

	% Atitikimas[a]			**% bendras atitikimas mėginyje**
Mėginio ID	**Vieta 1**	**Vieta 2**	**Vieta 3**	
Neigiamas	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toksigeniniam *C. difficile* stipriai neigiamas	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toksigeniniam *C. difficile* silpnai teigiamas	100% (20/20)	85% (17/20)	85% (17/20)	90% (54/60)

Toksigeniniam *C. difficile* vidutiniškai teigiamas	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toksigeniniam *C. difficile* ribotipui 027 High Negative	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Toksigeniniam *C. difficile* ribotipui 027 teigiamas	100% (20/20)	95% (19/20)	95% (19/20)	96.7% (58/60)
Toksigeniniam *C. difficile* ribotipui 027 vidutiniškai teigiamas	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
% Bendras atitikimas vietoje	100% (140/140)	97.1% (136/140)	97.1% (136/140)	98.1% (412/420)

a. Neigiamiems ir stipriai neigiamiems mėginiams, % atitikimas = (# neigiami rezultatai/visi mėginiai); silpnai ir vidutiniškai teigiamiems mėginiams, % atitikimas = (# teigiami rezultatai/visi mėginiai).

12 lentelė. Ct vertės rezultatų pagal mėginio lygį ir mėgintuvėlį santrauka

SPC			
Lygis	**Vid.**	**Stand.deviacija**	**CV**
Toksigeninis *C. diff* stipriai neig.	32.17	0.59	1.83%
Toksigeninis *C. diff* silpnai teig.	32.14	0.53	1.66%
Toksigeninis *C. diff* vid.teig.	31.98	0.47	1.47%
027 stipriai neig.	32.11	0.65	2.03%
027 silpnai teig.	31.93	0.72	2.26%
027 vid.teig.	31.96	0.61	1.90%
Neg	32.26	0.72	2.22%
***tcdB* (Toksinas B)**			
Lygis	**Vid.**	**Stand.deviacija**	**CV**
Toksigeninis *C. diff* high neg	39.59	0.70	1.77%
Toksigeninis *C. diff* silpnai teig.	35.88	0.81	2.24%
Toksigeninis *C. diff* vid.teig.	32.17	0.45	1.39%
027 stipriai neig.	39.11	0.98	2.50%
027 silpnai teig.	35.49	0.58	1.65%
027 vid.teig.	32.10	0.63	1.97%

Papildomas 6 mėginių panelis, trys neigiami ir trys toksigeniniam *C. difficile* stipriai teigiami mėginiai, buvo tiriami 5 skirtingas dienas, tyrimus atliko du skirtingi operatoriai, trijose skirtingose vietose (6 mėginiai x 2 operatoriai/diena x 5 dienos x 3 vietos). Stipriai neigiami mėginiai buvo paruošti ties koncentracija, žemesne nei LoD, tokiu būdu tikėtasi gauti neigiamą rezultatą 20 - 80% visų atvejų. Visose 3 tyrimo vietose buvo naudojama viena Xpert *C. difficile* BT tyrimo partija. Xpert *C. difficile* BT tyrimai buvo atliekami laikantis Xpert *C. difficile* BT tyrimo procedūrų. Rezultatų santrauka yra pateikiama 13 lentelėje.

13 lentelė. Papildomų mėginių atkuriamumo rezultatų santrauka

	% Atitikimas[a]			**% bendras atitikimas mėginyje**
Mėginio ID	**Vieta 1**	**Vieta 2**	**Vieta 3**	
Neigiamas	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)
Toksigeniniam *C. difficile* stipriai neigiamas[b]	60% (18/30)	60% (18/30)	53.3% (16/30)	57.8% (52/90)

a. (# neigiami rezultatai / visi stipriai neigiami mėginiai)
b. 20-80% tikėtinas atitikimas stipriai neigiamiems mėginiams

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22. Cepheid ofisas

Kompanijos būstinė	**Atstovybė Europoje**
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Tel. nr. +1 408.541.4191	Tel. nr. +33 563 825 300
Faks. +1 408.541.4192	Faks. +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

23. Techninė pagalba

Prieš susisiekiant su Cepheid techninės pagalbos skyriumi, turėkite šią informaciją:

- Produkto pavadinimas
- Partijos numeris
- Serijinis instrumento numeris
- Klaidų pranešima (jei yra)
- Programinės įrangos versija, ir, jei taikoma, kompiuterio priežiūros lipduko informacija

Regionas	**Telefono nr.**	**El.paštas**
JAV – Techninė pagalba	+1 888.838.3222	techsupport@cepheid.com
Australija ir Naujoji Zelandija	1800 107 884 0800 001 028	techsupportanz@cepheid.com
Kinija	+86 021 5406 5387	techsupportchina@cepheid.com
Prancūzija	+33 563 825 319	support@cepheideurope.com
Vokietija	+49 69 710 480 480	support@cepheideurope.com
Jungtinė Karalystė	+44 3303 332 533	support@cepheideurope.com
Italija	+39 800 902 567	support@cepheideurope.com
Pietų Afrika	+27 861 22 76 35	support@cepheideurope.com

Kitos Europos, Vidurio Rytų ir Afrikos šalys	+33 563 825 319 +971 4 253 3218	support@cepheideurope.com
Japonija	+0120 95 4886	support@japan.cepheid.com
Nepaminėtos šalys	+1 408.400.8495	techsupport@cepheid.com

Kontaktinę kitų Cepheid ofisų informaciją rasite tinklalapyje www.cepheid.com, www.cepheidjapan.com ar www.cepheidinternational.com **SUPPORT** (pagalba) skirtuke. Pasirinkite **Contact Us** (susisiekite) parinktį.

24. Simbolių lentelė

Simbolis	Reikšmė
REF	Katalogo numeris
IVD	*In vitro* diagnostinė medicinos priemonė
2	Vienkartinio naudojimo
LOT	Partijos kodas
	Skaitykite naudojimo instrukcijas
	Dėmesio
	Gamintojas
	Turinys skirtas <n> tyrimams
CONTROL	Kontrolė
	Galiojimo data
CE	CE žyma – Europos atitiktis
EC REP	Įgaliotas atstovas Europos Bendrijoje
	Temperatūros apribojimai
	Biologinis pavojus
	Įspėjimas

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Švedija

IVD CE

Tikslus dokumento vertimas į lietuvių kalbą
Vertėja Akvilė Gegelevičienė
Data 2017-06-08

UAB Diamedica
Molėtų pl. 73, Vilnius, Lietuva
Tel. 8 5 279 0080

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Flu/RSV XC

REF **GXFLU/RSV-CE-10**

Cepheid

In Vitro Diagnostic Medical Device CE IVD

301-2904, Rev. B September 2015

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Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA
www.cepheid.com

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France
www.cepheidinternational.com/

Xpert® Flu/RSV XC

For *In Vitro* Diagnostic Use Only.

1 Proprietary Name

Xpert® Flu/RSV XC

2 Common or Usual Name

Xpert Flu/RSV XC Assay

3 Intended Use

The Cepheid Xpert Flu/RSV XC Assay, performed on the GeneXpert Instrument Systems, is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA. The Xpert Flu/RSV XC Assay uses nasopharyngeal swab and nasal aspirate/wash specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Flu/RSV XC Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus infections in conjunction with clinical and epidemiological risk factors.

Negative results do not preclude influenza virus or respiratory syncytial virus infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2013–2014 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

4 Summary and Explanation

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (*i.e.*, coughing or sneezing); the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, muscle aches, malaise, cough, and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting, or diarrhea) may also occur, primarily in children, but are less common in adults. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication of influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[1,2]

Influenza viruses are classified into types A, B, and C, the former two of which cause most human infections. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and occasionally for pandemics. Influenza A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B virus are generally restricted to humans and are less frequent causes of epidemics. Influenza A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by subtypes H1, H2, H3, and N1 and N2. In addition to seasonal flu, a novel H1N1 strain was identified in humans in the United States in early 2009.[3]

Respiratory syncytial virus (RSV), a member of the Paramyxoviridae family consisting of two strains (subgroups A and B), is also the cause of a contagious disease that afflicts primarily infants and the elderly who are immunocompromised, e.g., patients with chronic lung or heart disease or undergoing treatment for conditions that reduces the strength of their immune system.[3] The virus can live for hours on countertops and toys and causes both upper respiratory infections, such as tracheobronchitis and lower respiratory infections manifesting as bronchiolitis and pneumonia.[4] By the age of two, most children have already been infected by RSV, but because only weak immunity develops, both children and adults can become reinfected.[3] Symptoms usually appear four to six days after infection. The disease is typically self-limiting, lasting about one to two weeks in infants. In adults, the infection lasts about five days and presents with symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season overlaps with influenza season somewhat as infections begin to rise during the fall and continues through early spring.[3,4] RSV infections, however, also occur at other times of the year, although rarely.

Active surveillance programs in conjunction with infection control precautions are important components for preventing transmission of influenza and RSV. The use of assays providing rapid results to identify patients infected with these seasonal infections is also an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

5 Principle of the Procedure

The Xpert Flu/RSV XC Assay is an automated *in vitro* diagnostic test for qualitative detection of influenza A, influenza B, and RSV. The assay is performed on Cepheid GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample extraction, purification, amplification, and detection of nucleic acid target sequences in clinical specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. Each test requires the use of a single-use disposable GeneXpert cartridge that contains target-specific reagents and carries out the RT-PCR and PCR processes. Because the cartridges are self-contained, the risk of cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx System Operator Manual* or *GeneXpert Infinity System Operator Manual*.

The Xpert Flu/RSV XC Assay includes reagents for the detection and differentiation of influenza A, influenza B, and RSV viral RNA directly from nasal aspirate/wash (NA/W) specimens and nasopharyngeal (NP) swab specimens from patients with signs and symptoms of respiratory infection. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate extraction and processing of the target sequences and to monitor for the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Xpert Flu/RSV XC Assay can be run to detect Flu A and Flu B only by selecting **Xpert Flu XC**; RSV only by selecting **Xpert RSV**; or Flu A, Flu B, and RSV by selecting **Xpert Flu-RSV XC** from the **Select Assay** menu. Xpert Flu XC and Xpert RSV Assays have an Early Assay Termination (EAT) function that enables early result reporting. EAT is activated when the pre-determined threshold for a positive test result is reached before the full 40 PCR cycles have been completed. When Flu A or Flu B viral titers are high enough to generate very early Cts with the Xpert Flu XC Assay, SPC amplification curves will not be seen and its results will not be reported. When RSV titers are high enough to generate very early Cts with the Xpert RSV Assay, SPC amplification curves will not be seen and its results will not be reported.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Flu/RSV XC Assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Flu/RSV XC Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent (Guanidinium Thiocyanate)	1.5 mL per cartridge
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
Disposable 300 µL Transfer Pipettes	**2 bags of 12 per kit**
CD	**1 per kit**
• Assay Definition Files (ADF)	
• Instructions to import ADF into GeneXpert software	
• Package Insert	

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

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

7 Storage and Handling

- Store the Xpert Flu/RSV XC Assay cartridges at 2–28 °C. until the expiration date provided on the package label.
- Do not open a cartridge lid until you are ready to perform testing.

- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- Specimens must be collected and transported with the Xpert Nasopharyngeal Sample Collection Kit for viruses, Cepheid catalog #SWAB/B-100 or Sample Collection Device catalog #NASL-100N-100.
- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): six-color GeneXpert instrument, computer with proprietary GeneXpert Software version 4.3 or higher, barcode scanner, and operator manual.
- Printer: Contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

9 Materials Available but Not Provided

- Inactivated virus controls from ZeptoMetrix, catalog #NATFLUAB-6C and catalog #NATRSV-6C as external positive controls, and catalog #NATCXVA9-6C (Coxsackie virus) as an external negative control.

10 Warnings and Precautions

10.1 General

- For *In Vitro* Diagnostic Use Only.

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[5] and the Clinical and Laboratory Standards Institute.[6,7]
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
- Performance characteristics of this test have been established with the specimen types listed in the Intended Use section only. The performance of this assay with other specimen types or samples has not been evaluated.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

- Lysis Reagent contains Guanidinium thiocyanate (H302, H316, H320, H402, EUH031), which is harmful to aquatic life and contact with acids liberates toxic gas.

10.2 Specimen

- Specimen collection and handling procedures require specific training and guidance.
- For collection and transport of nasopharyngeal swab specimens, use only the Xpert Nasopharyngeal Sample Collection Kit.
- Specimens must be collected and tested before the expiration date of the Xpert Viral Transport Medium tube.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 11, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Proper sample collection, storage, and transport are essential for correct results.

10.3 Assay/Reagent

- The assay has been validated using Cepheid GeneXpert software version 4.3 or higher. Cepheid will validate future software versions for use with the Xpert Flu/RSV XC Assay.
- Use of the influenza A/influenza B positive control in the Xpert RSV only assay mode may lead to invalid control results.
- Use of the RSV positive control in the Xpert Flu XC only assay mode may lead to invalid control results.
- When performing a test in the Xpert Flu XC only or Xpert RSV only mode, a negative test result does not preclude a positive result for the other targets.
- Sensitivity may be impacted when using frozen, archived specimens.
- Do not open the Xpert Flu/RSV XC Assay cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Flu/RSV XC Assay cartridge is used to process one test. Do not reuse spent cartridges, except when diluting NA/W specimens.
- Each 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.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- Wear clean lab coats and gloves. In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a 1:10 dilution of household chlorine bleach and then 70% denatured ethanol. Wipe work surfaces dry completely before proceeding.

11 Specimen Collection, Transport, and Storage

NA/W specimens and NP swab specimens can be collected following the user institution's standard procedures and placed into the Xpert Viral Transport Medium (3 mL tube with transport medium). Specimens should be transported at 2–8 °C.

- Specimens placed in transport medium following collection can be stored for up to 24 hours at 2–30 °C or up to seven days at 2–8 °C prior to testing with the Xpert Flu/RSV XC Assay.

Proper specimen collection, storage, and transport are critical to the performance of this test.

12 Procedure

12.1 Preparing the Cartridge

Important **Start the test within 60 minutes of adding the sample to the cartridge.**

For NP Swab Specimens:

1. Remove a cartridge from the package.
2. Mix specimen by inverting the Xpert Viral Transport Medium tube five times.
3. Open the cartridge lid. Using a clean 300 µL transfer pipette (supplied), transfer 300 µL (one draw) of the specimen from the transport medium tube to the sample chamber with large opening in the cartridge (Figure 1).
4. Close the cartridge lid.

For NA/W Specimens:

1. Using a clean 300 µL transfer pipette (supplied), transfer 600 µL of the sample (two draws, using the same transfer pipette) into the 3 mL Xpert Viral Transport Medium tube and then cap the tube.
2. Mix specimen by inverting the transport medium tube five times.
3. Remove the cartridge from the package.
4. Open the cartridge lid. Using a clean 300 µL transfer pipette (supplied), transfer 300 µL (one draw) of the diluted specimen to the sample chamber with the large opening in the cartridge (Figure 1).
5. Close the cartridge lid.

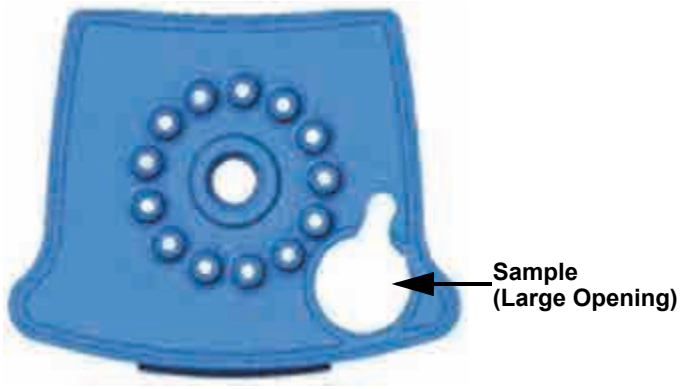

Figure 1. Xpert Flu/RSV XC Assay Cartridge (Top View)

12.2 Starting the Test

Important **Before starting the test, make sure the Xpert Flu/RSV XC Assay definition files (ADFs) are imported into the software.**

This section lists the basic steps for running the test. 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:
   - 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 icon on the Windows® desktop.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. The GeneXpert software will launch automatically or may require double-clicking the Xpertise software icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (GeneXpert Infinity). The Create Test window opens.
4. Scan (or type in) the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test results.
5. Scan (or type in) the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test results.
6. Scan the barcode on the Xpert Flu/RSV XC Assay cartridge. Using the barcode information, the software automatically fills in the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert Flu/RSV XC Assay cartridge does not scan, then repeat the test with a new cartridge.

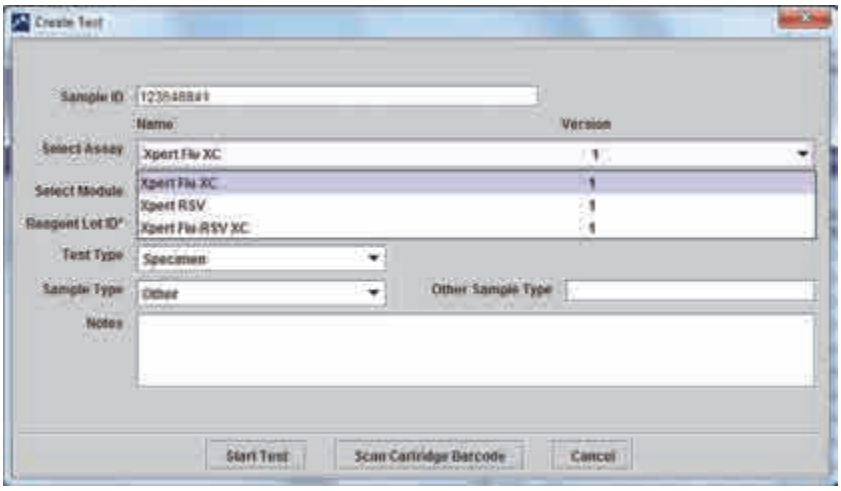

Figure 2. Create Test Window: Select Assay Menu

7. Make the appropriate selection from the **Select Assay** menu, as shown in Figure 2.
   - Flu A and Flu B only: Select **Xpert Flu XC**
   - RSV only: Select **Xpert RSV**
   - Flu A, Flu B and RSV: Select **Xpert Flu-RSV XC**

Note Only the test result for the assay selected at this step will be collected once the test is started. Flu A, Flu B, and RSV results will only be collected if the Xpert Flu-RSV XC assay is chosen.

8. Click **Start Test** (GeneXpert Dx) or **Submit** (GeneXpert Infinity). Type your password in the dialog box that appears.
9. 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 and removing the cartridge.
   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

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 upon 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

14.1 Built-in Quality Controls

CONTROL

Each test includes a Sample Processing Control (SPC) and a Probe Check Control (PCC).

- **Sample Processing Control (SPC):** Ensures the sample was processed correctly. The SPC is an Armored RNA® that is included in each cartridge to verify adequate processing of the sample. The SPC verifies that release of RNA from the influenza and RSV viruses has occurred if the organism is present and verifies that the specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the RT-PCR and PCR reactions. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

  There are two exceptions in which SPC is ignored and the result is valid:

  - The SPC may be negative in a sample with a high titer of Flu A or Flu B when tested with the Xpert RSV ADF.
  - The SPC may be negative in a sample with a high titer of RSV when tested with the Xpert Flu XC ADF.

- **Probe Check Control (PCC, QC1, QC2):** Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the first PCC (QC1 and QC2) performed before the reverse transcription step. QC1 checks for the presence of the EZR bead and QC2 checks for the presence of the TSR bead. The second PCC (Flu A 1, Flu A 2, Flu A 3, Flu B, RSV, and SPC) is performed after the reverse transcription step and before PCR begins. The Probe Check Control (PCC, QC1 and QC2) monitors bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

15 Interpretation of Results

The Xpert Flu/RSV XC Assay has three channels (Flu A 1, Flu A 2, and Flu A 3) to detect most influenza A strains. The primers and probes in the Flu A 1 channel have 100% homology to human influenza A strains. The primers and probes in the Flu A 2 channel have > 95% homology to avian influenza A strains and approximately 80% homology to human influenza A strains. The primers and probes in the Flu A 3 channel detect the hemagglutinin gene segment for the avian influenza A H7N9 strains (subtyping capability). All influenza A strains (human and avian) detected by the Xpert Flu/RSV XC Assay are reported as **Flu A POSITIVE**.

The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window. A Flu A result in the Xpert Flu/RSV XC Assay requires either the Flu A 1 or Flu A 2 channel to be positive in order for a **Flu A POSITIVE** test result to be reported. A positive in the Flu A 3 channel without a positive Flu A 1 or Flu A 2 result is reported as **INVALID**. Table 1 lists all the possible test results for Flu A.

Table 1. Possible Test Results for Flu A for Flu A 1, Flu A 2, and Flu A 3 Channels

Flu A Test Result	Flu A 1 Channel	Flu A 2 Channel	Flu A 3 Channel
Flu A POSITIVE	POS	POS	POS
	POS	POS	NEG
	POS	NEG	POS
	NEG	POS	POS
	POS	NEG	NEG
	NEG	POS	NEG
INVALID	NEG	NEG	POS
Flu A NEGATIVE	NEG	NEG	NEG

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. All the possible results are shown in Table 2.

Table 2. All Possible Final Test Results for the Xpert Flu-RSV XC Selected Assay

Result Text	Flu A 1	Flu A 2	Flu A 3	Flu B	RSV	SPC
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE	+/-	+/-	+/-	-	-	+/-
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE	-	-	-	+	-	+/-
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE	-	-	-	-	+	+/-
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE	+/-	+/-	+/-	+	-	+/-
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE	+/-	+/-	+/-	-	+	+/-
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE	-	-	-	+	+	+/-
Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE	+/-	+/-	+/-	+	+	+/-
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	-	-	-	-	-	+
INVALID	-	-	-	-	-	-

See Figure 3 through Figure 19 for specific examples and Table 3 to interpret test result statements for the Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV assays. The format of the test results presented will vary depending on the user's choice to run either an Xpert Flu-RSV XC, Xpert Flu XC, or Xpert RSV selected assay.

Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations

Result	Interpretation
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE See Figure 3.	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu A target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE See Figure 4.	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is not detected. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu B target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE See Figure 5.	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is detected. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the RSV target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.

Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations (Continued)

Result	Interpretation
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE See Figure 6.	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu A and Flu B target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE See Figure 7.	Flu A target RNA is detected; Flu B target RNA is not detected; RSV target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu A and RSV target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE See Figure 8.	Flu A target RNA is not detected; Flu B target RNA is detected; RSV target RNA is detected. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu B and RSV target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE See Figure 9.	Flu A target RNA is detected; Flu B target RNA is detected; RSV target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu A, Flu B, and RSV target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE See Figure 10.	Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. • Flu A, Flu B, and RSV target RNAs are not detected. • SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting. • Probe Check: PASS; all probe check results pass.
INVALID See Figure 11.	• SPC does not meet acceptance criteria. Presence or absence of the target RNAs cannot be determined. Repeat test according to the instructions in Section 16.2, Retest Procedure. • SPC meets acceptance criteria. Flu A 1, Flu A 2, Flu B, and/or RSV target RNAs are not detected; FluA3 target RNA is detected.
Flu A POSITIVE; Flu B NEGATIVE See Figure 12.	Flu A target RNA is detected; Flu B target RNA is not detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu A target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B POSITIVE See Figure 13.	Flu A target RNA is not detected; Flu B target RNA is detected. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu B target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.

Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations (Continued)

Result	Interpretation
Flu A POSITIVE; Flu B POSITIVE See Figure 14.	Flu A target RNA is detected; Flu B target RNA is detected. • The Flu A target has a Ct within the valid range and endpoint above the threshold setting. • The Flu B target has a Ct within the valid range and endpoint above the threshold setting. • SPC: NA (not applicable); SPC is ignored because the Flu A and Flu B target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
Flu A NEGATIVE; Flu B NEGATIVE See Figure 15 and Figure 16.	Flu A target RNA is not detected; Flu B target RNA is not detected (see Figure 15). • Flu A and Flu B target RNAs are not detected. • SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting. • Probe Check: PASS; all probe check results pass. Or Flu A target RNA is not detected (see Figure 16); Flu B target RNA is not detected (see Figure 16). • Flu A and Flu B target RNAs are not detected. • SPC: NA (not applicable); SPC is ignored because the RSV target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
RSV POSITIVE See Figure 17.	RSV target RNA is detected. • The RSV target has a Ct within the valid range and endpoint above the threshold setting. • SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting. • Probe Check: PASS; all probe check results pass.
RSV NEGATIVE See Figure 18 and Figure 19.	RSV target RNA is not detected (see Figure 18). • RSV target RNA is not detected. • SPC: PASS; SPC has a Ct within the valid range and endpoint above the threshold setting. • Probe Check: PASS; all probe check results pass. Or RSV target RNA is not detected (see Figure 19). • The RSV target RNA is not detected. • SPC: NA (not applicable); SPC is ignored because the Flu A or Flu B target amplification may compete with this control. • Probe Check: PASS; all probe check results pass.
ERROR	Presence or absence of Flu A, Flu B, and/or RSV target RNA cannot be determined. Repeat test according to the instructions in Section 16.2, Retest Procedure below. • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: FAIL*; all or one of the probe check results fail. * If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.

Table 3. Xpert Flu-RSV XC, Xpert Flu XC, and Xpert RSV Assay Results and Interpretations (Continued)

Result	Interpretation
NO RESULT	Presence or absence of Flu A, Flu B, and/or RSV target RNA cannot be determined. Repeat test according to the instructions in Section 16.2, Retest Procedure below. 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. • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

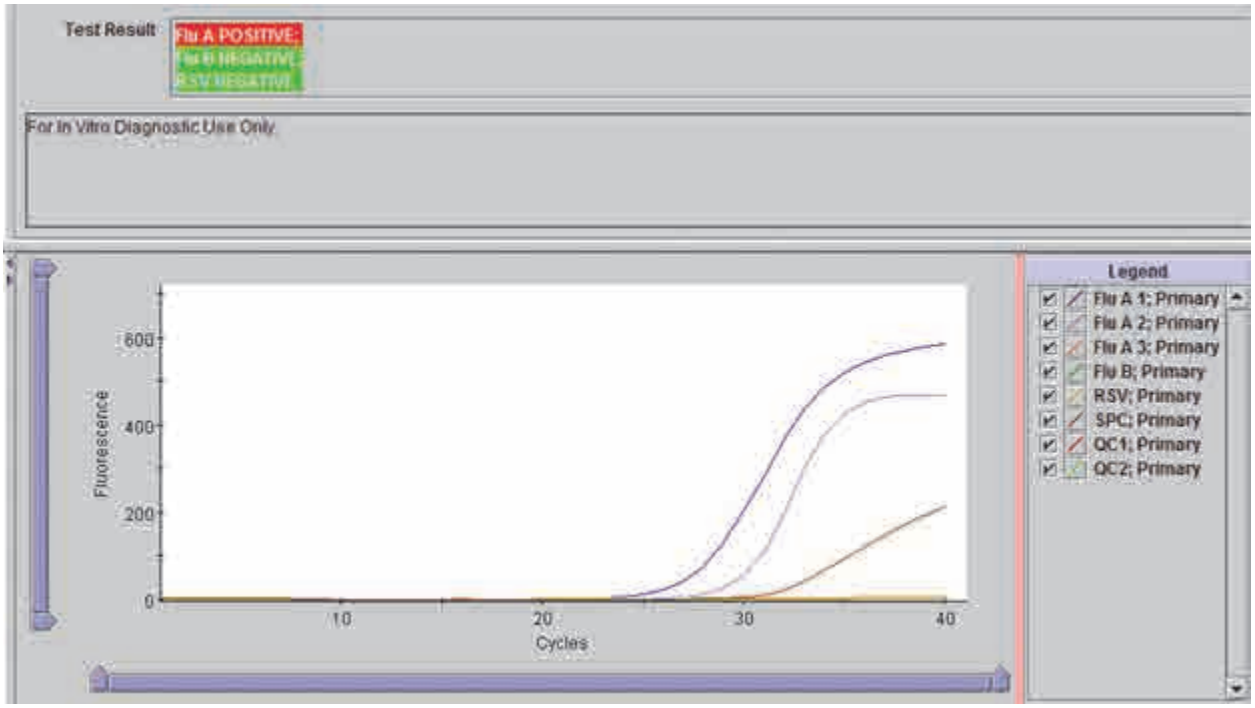

Figure 3. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A

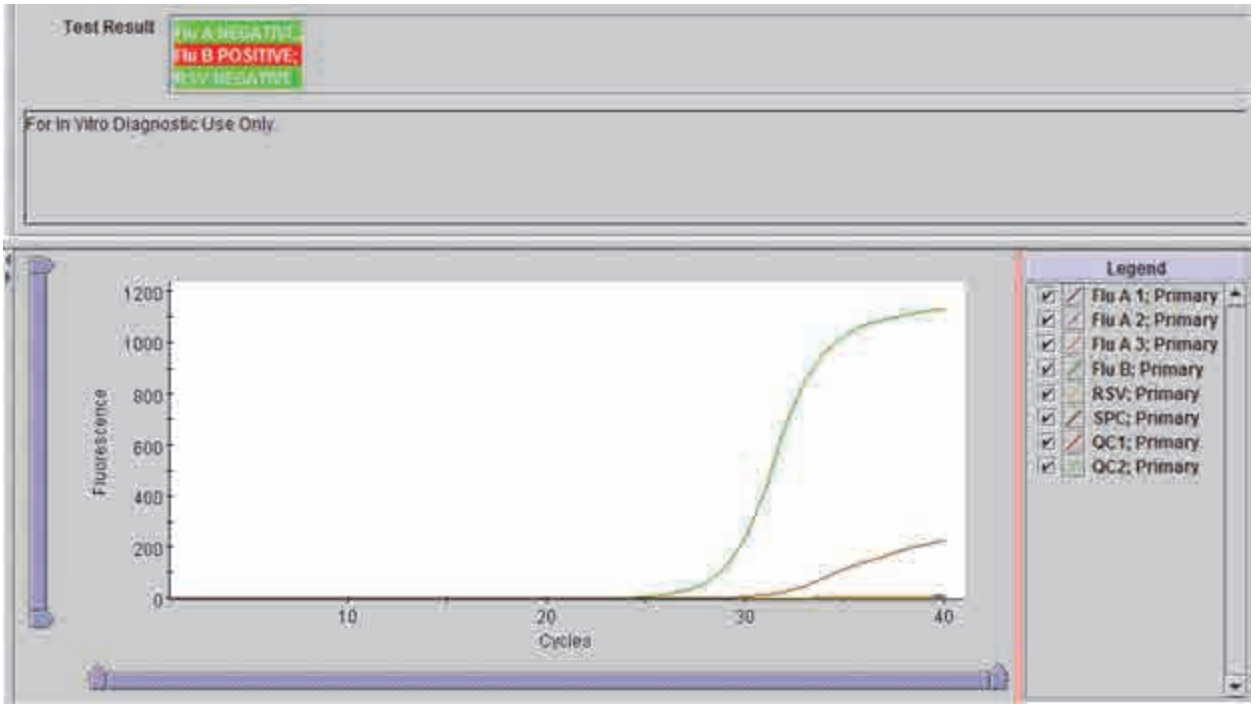

Figure 4. Xpert Flu-RSV XC: An Example of a Positive Result for Flu B

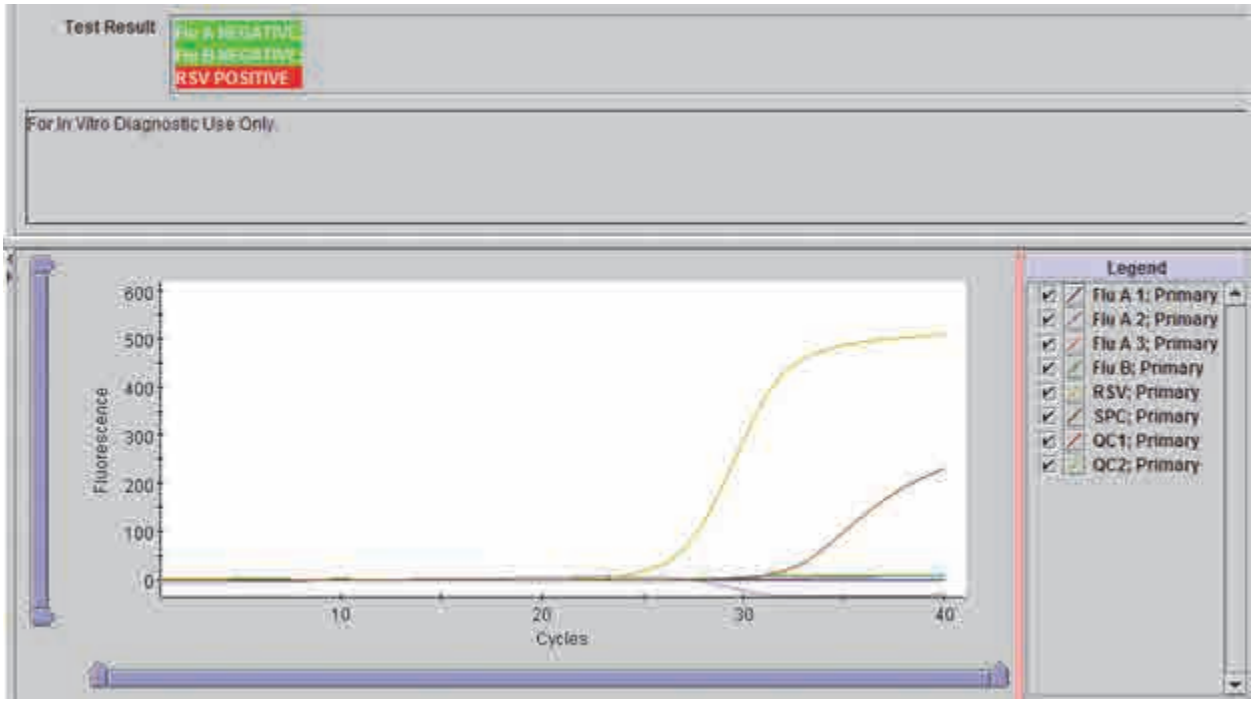

Figure 5. Xpert Flu-RSV XC: An Example of a Positive Result for RSV

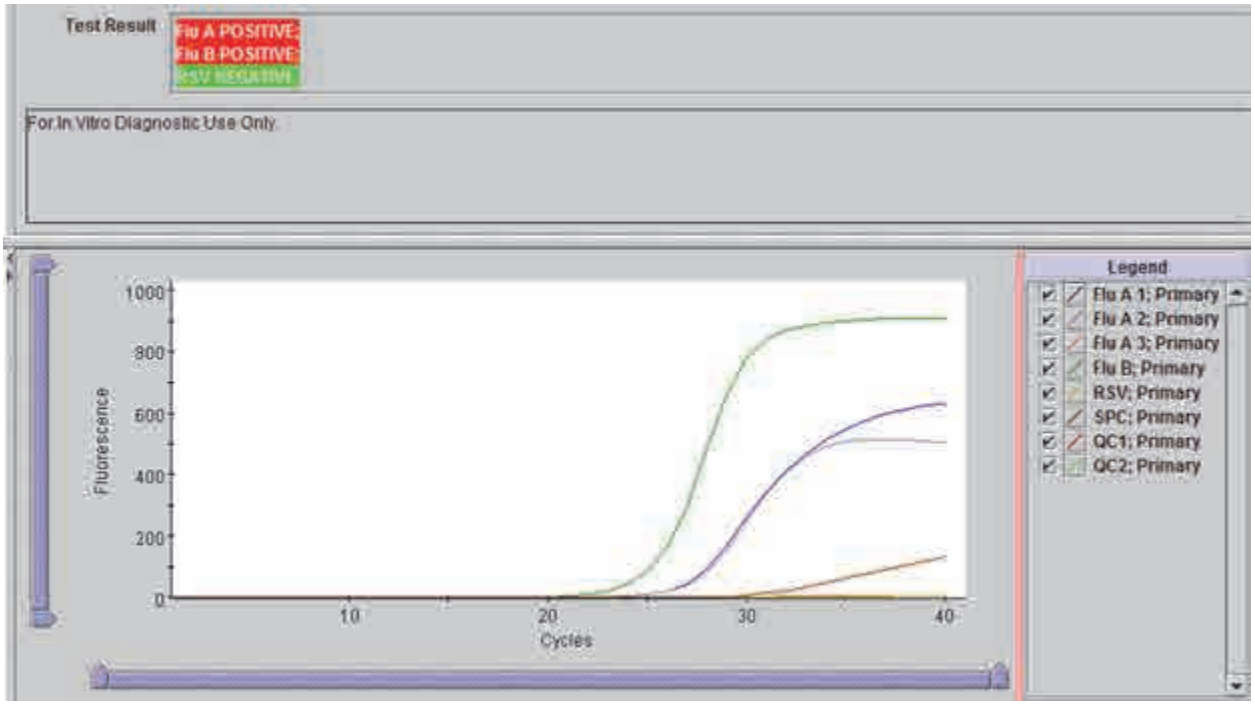

Figure 6. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A and Flu B

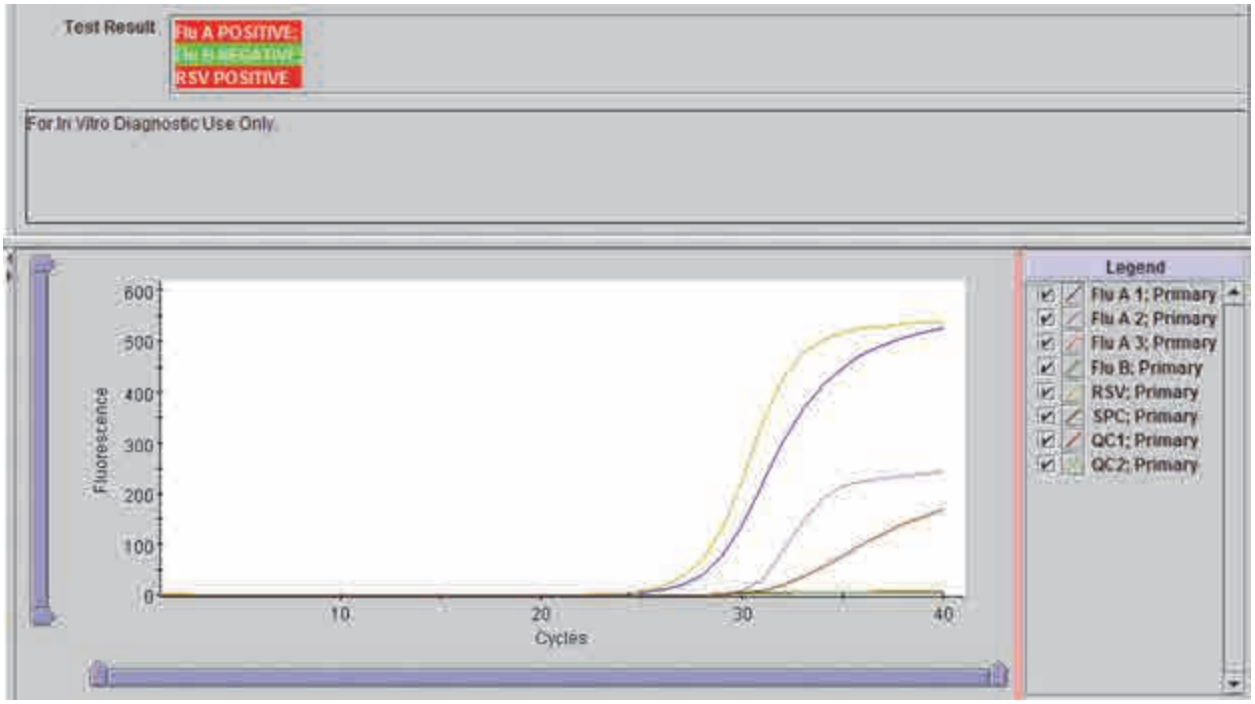

Figure 7. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A and RSV

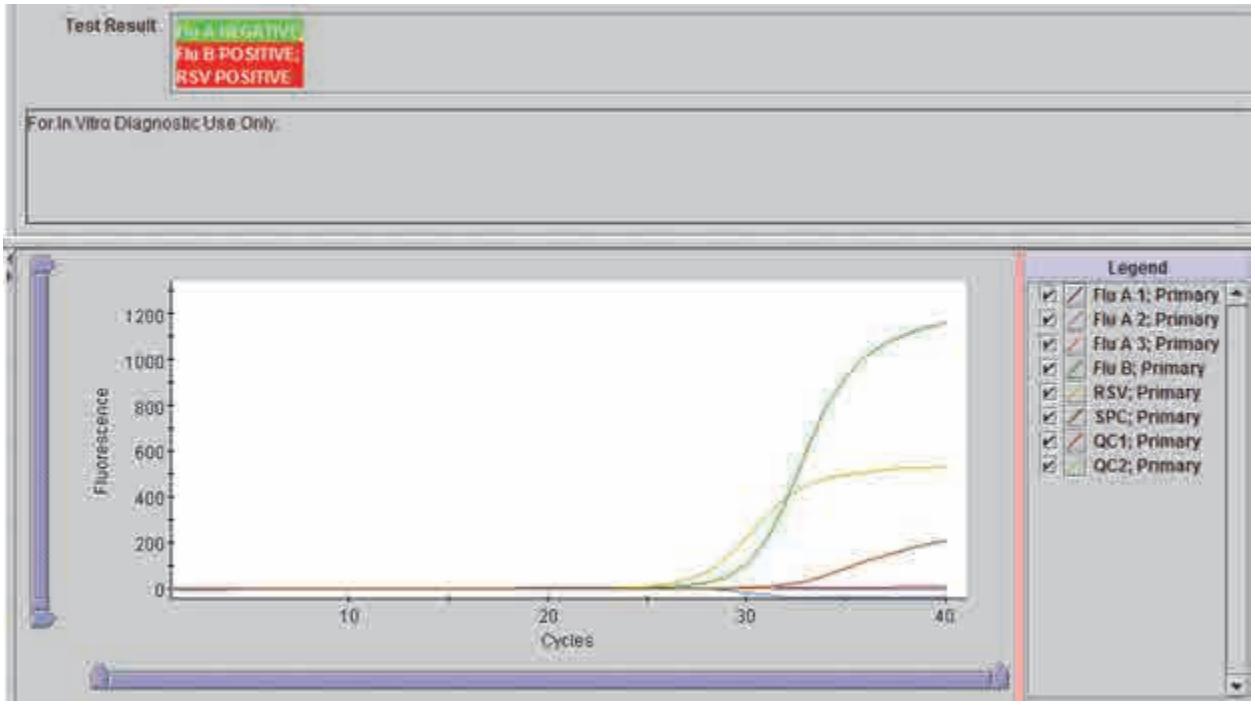

Figure 8. Xpert Flu-RSV XC: An Example of a Positive Result for Flu B and RSV

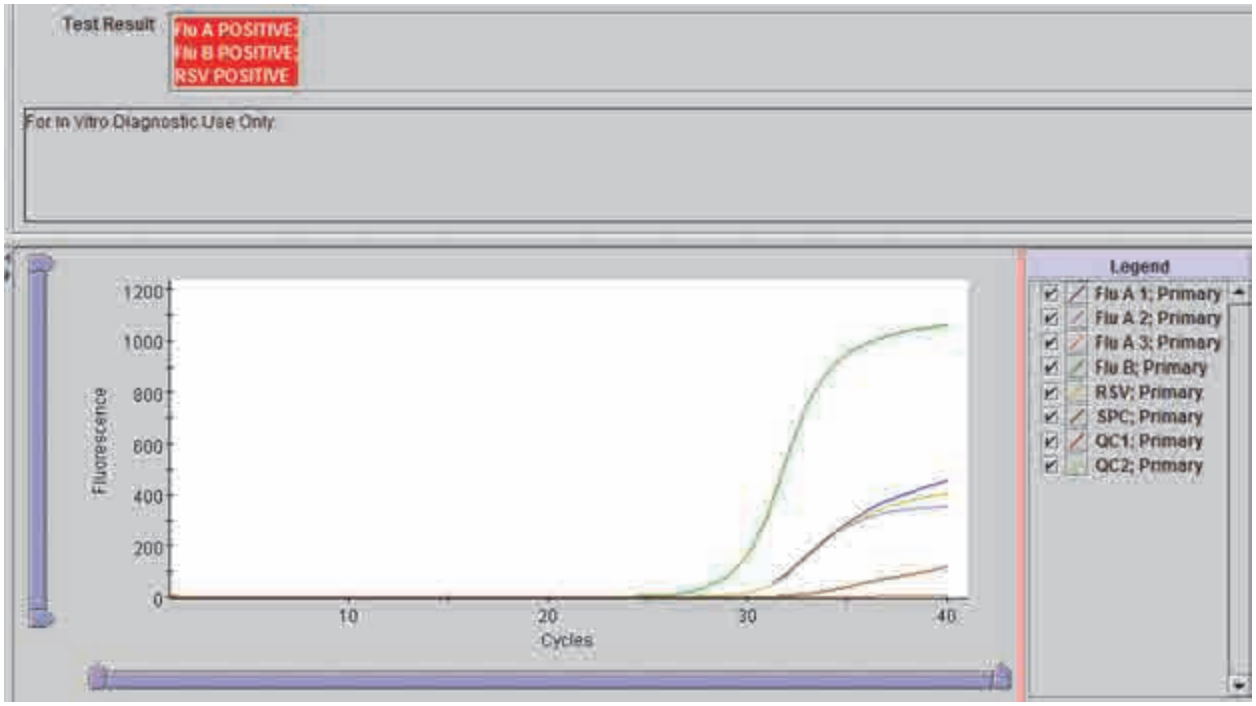

Figure 9. Xpert Flu-RSV XC: An Example of a Positive Result for Flu A, Flu B, and RSV

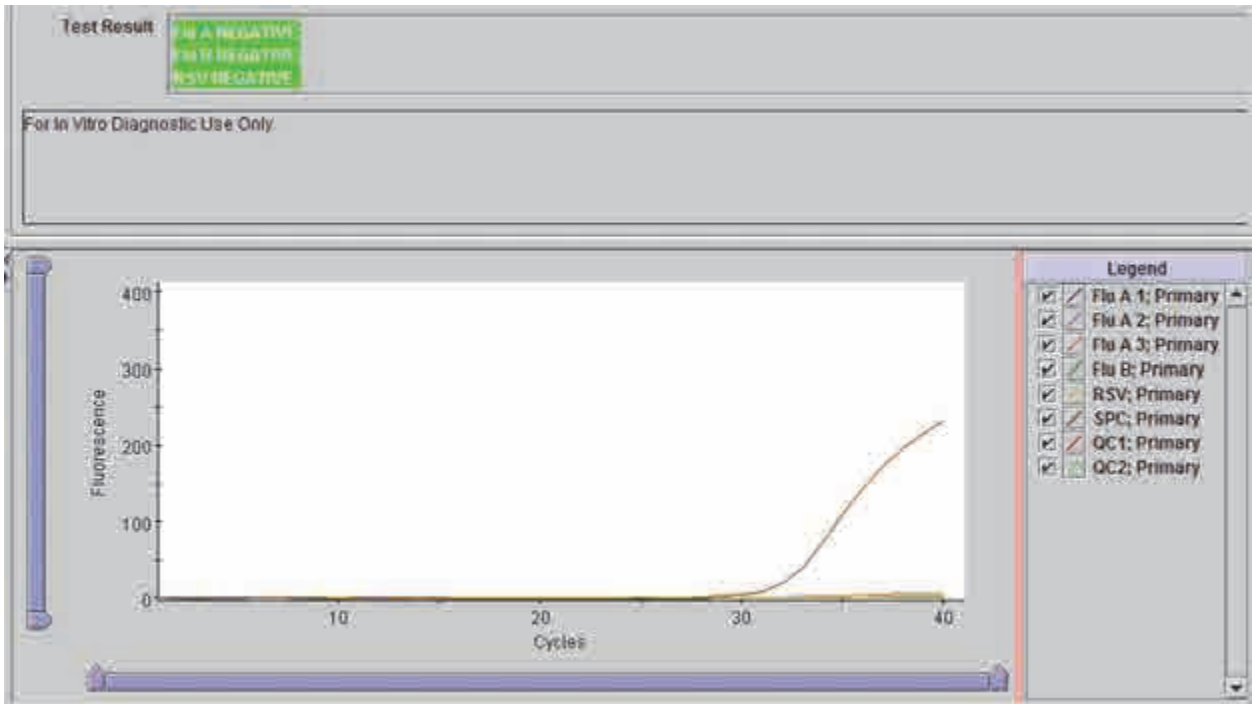

Figure 10. Xpert Flu-RSV XC: An Example of a Negative Result for Flu A, Flu B, and RSV

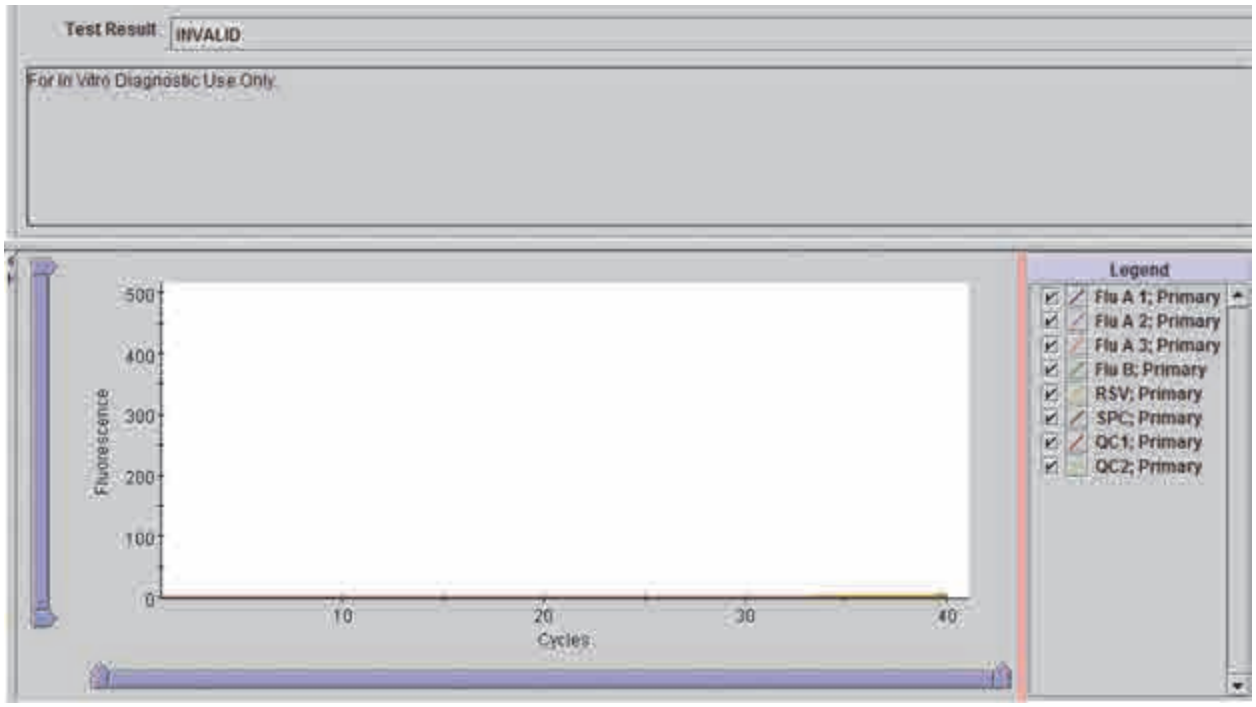

Figure 11. Xpert Flu-RSV XC: An Example of an Invalid Result (SPC does not meet Acceptance Criteria)

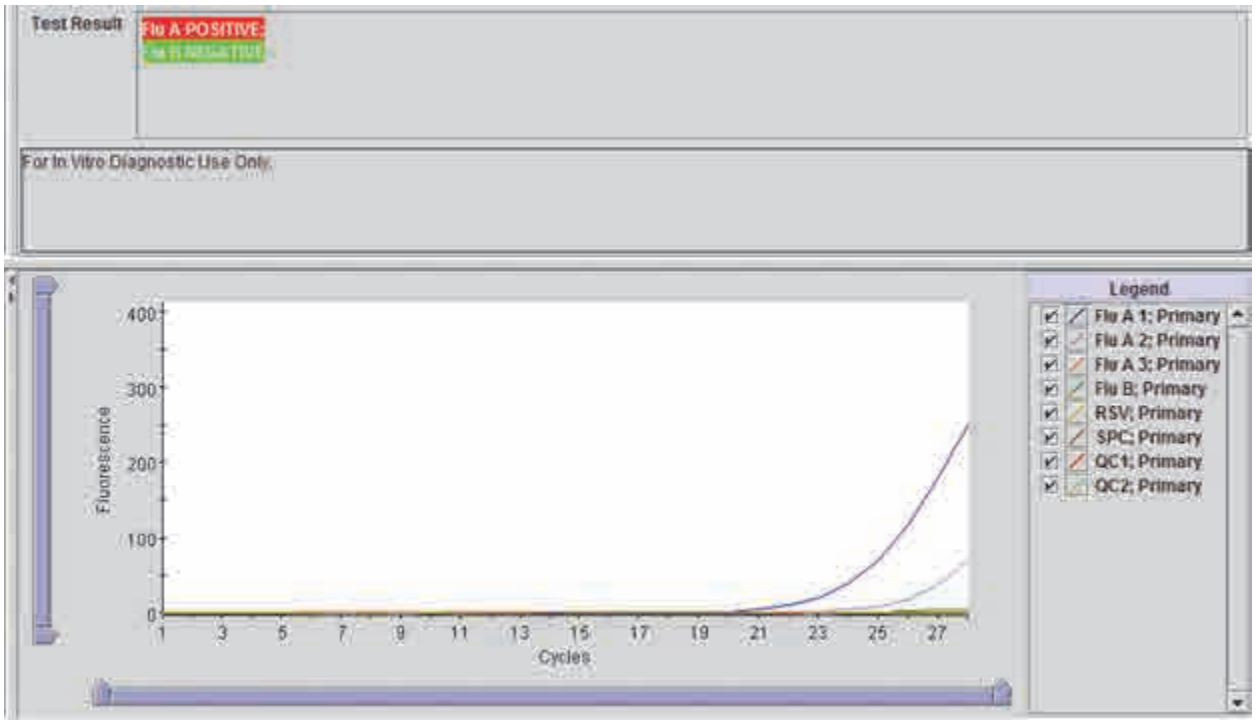

Figure 12. Xpert Flu XC: An Example of a Positive Result for Flu A

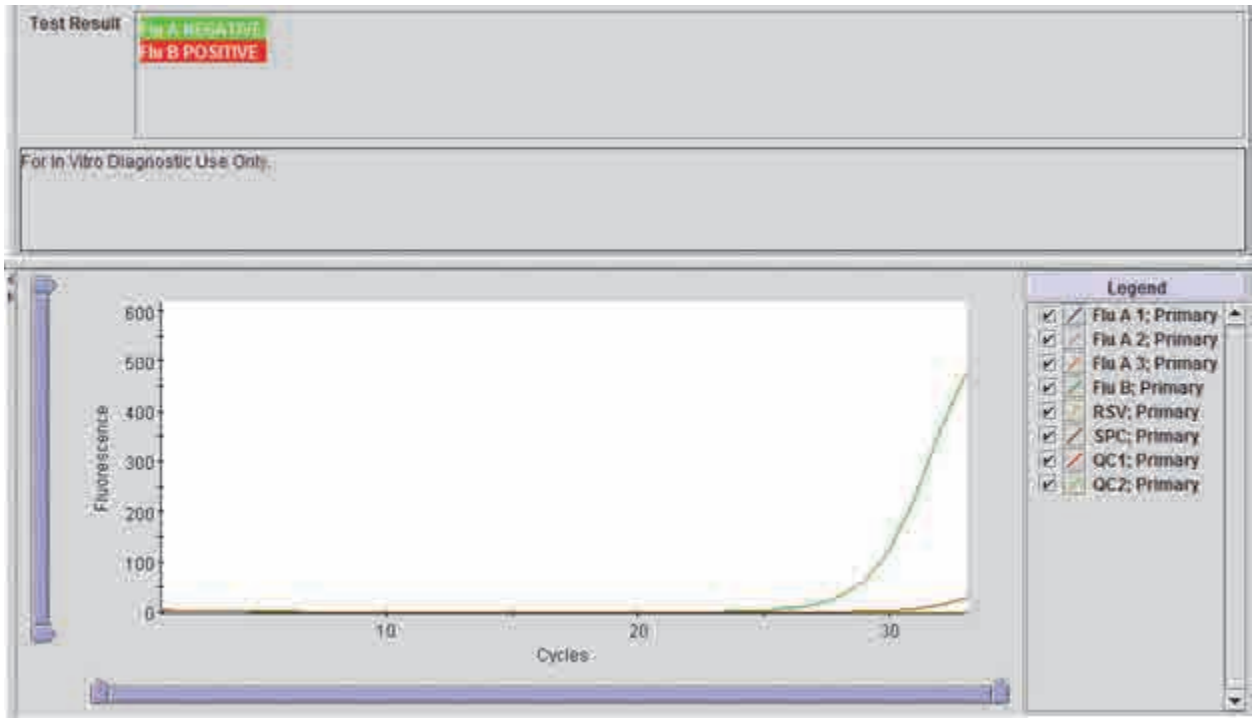

Figure 13. Xpert Flu XC: An Example of a Positive Result for Flu B

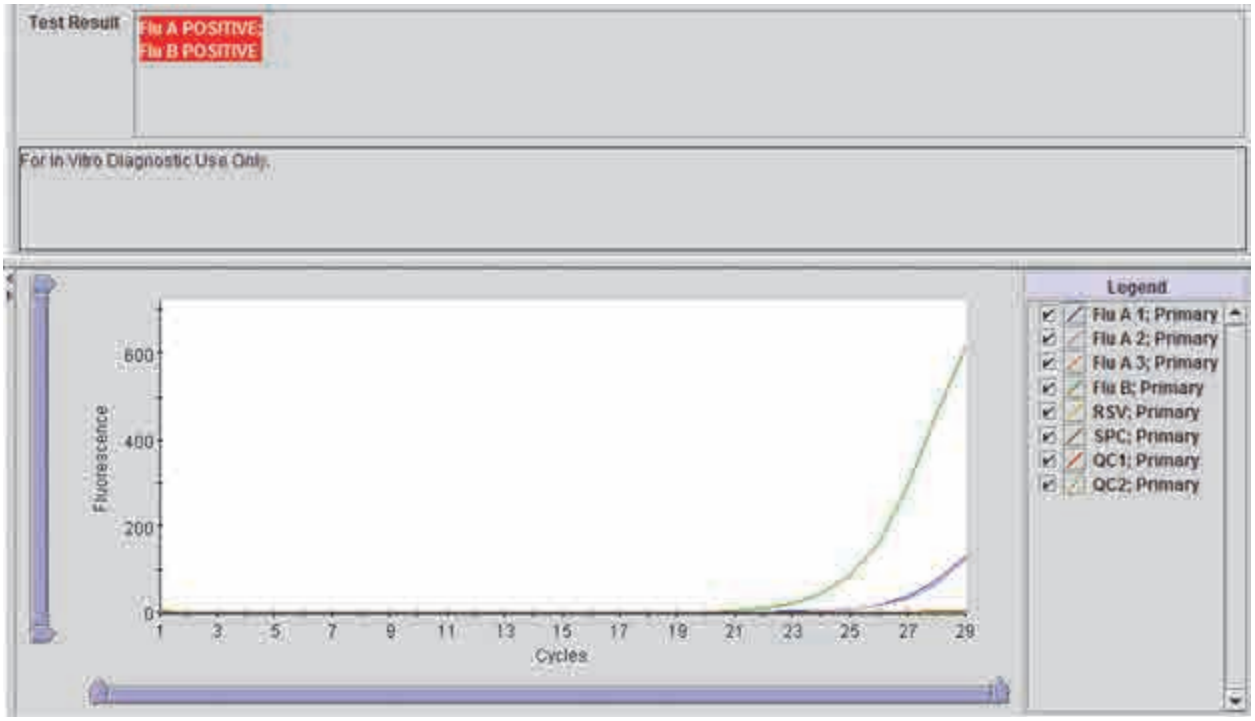

Figure 14. Xpert Flu XC: An Example of a Positive Result for Flu A and Flu B

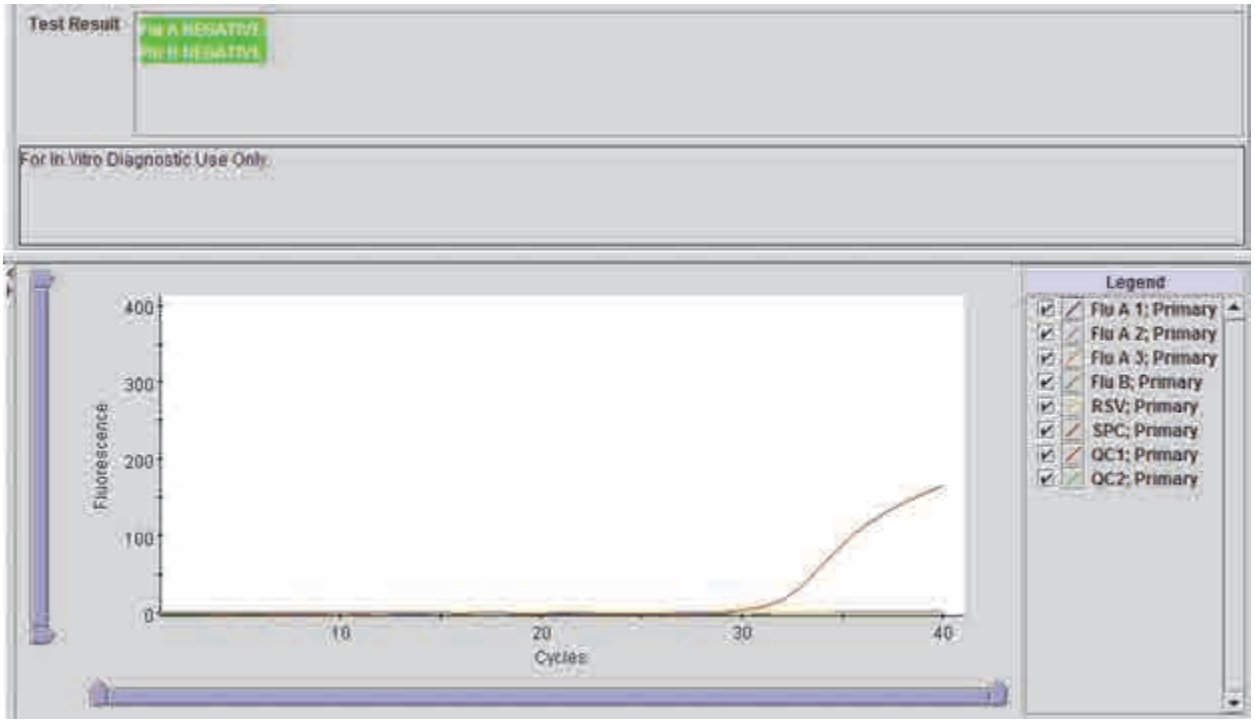

Figure 15. Xpert Flu XC: An Example of a Negative Result for Flu A and Flu B

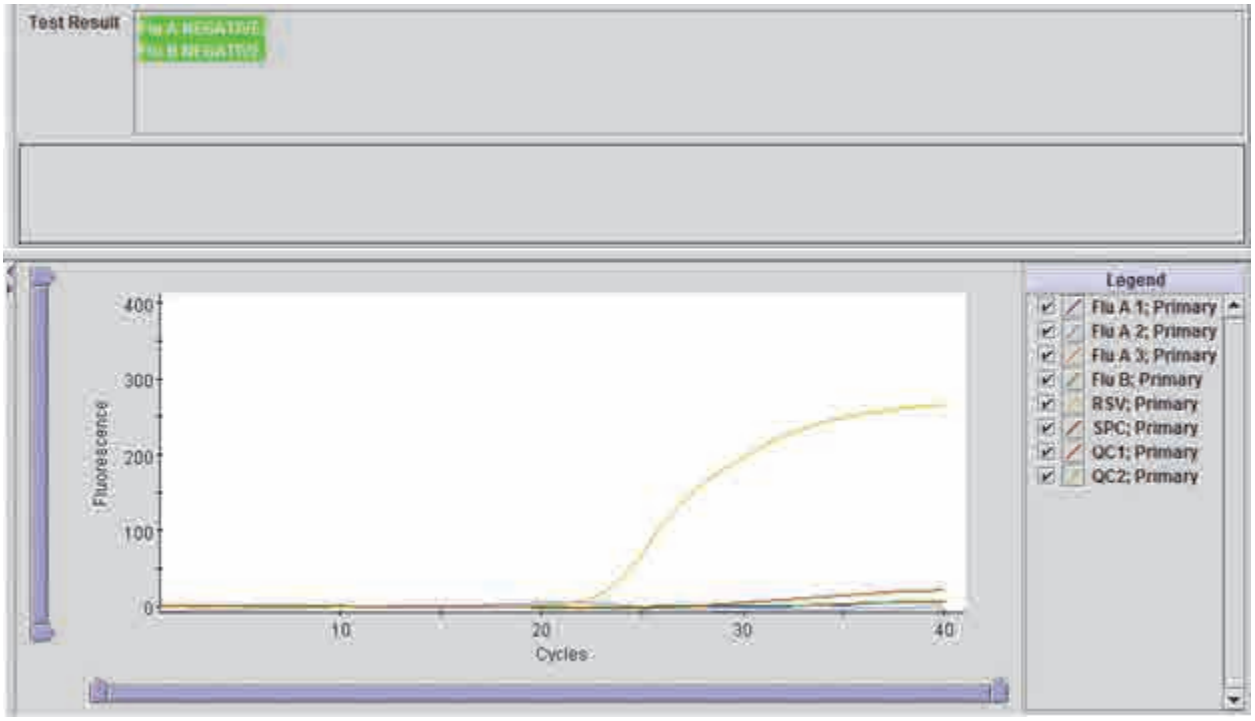

Figure 16. Xpert Flu XC: An Example of a Negative Result for Flu A and Flu B (Sample Containing RSV Target)

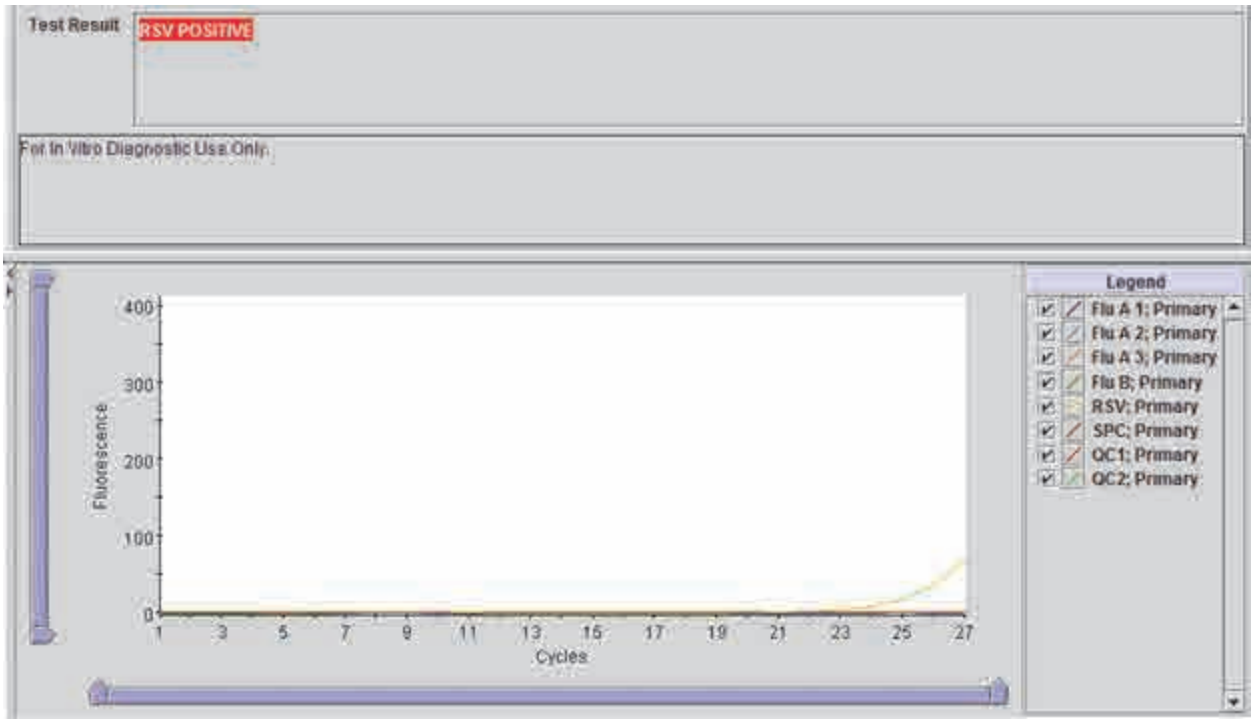

Figure 17. Xpert RSV: An Example of a Positive Result for RSV

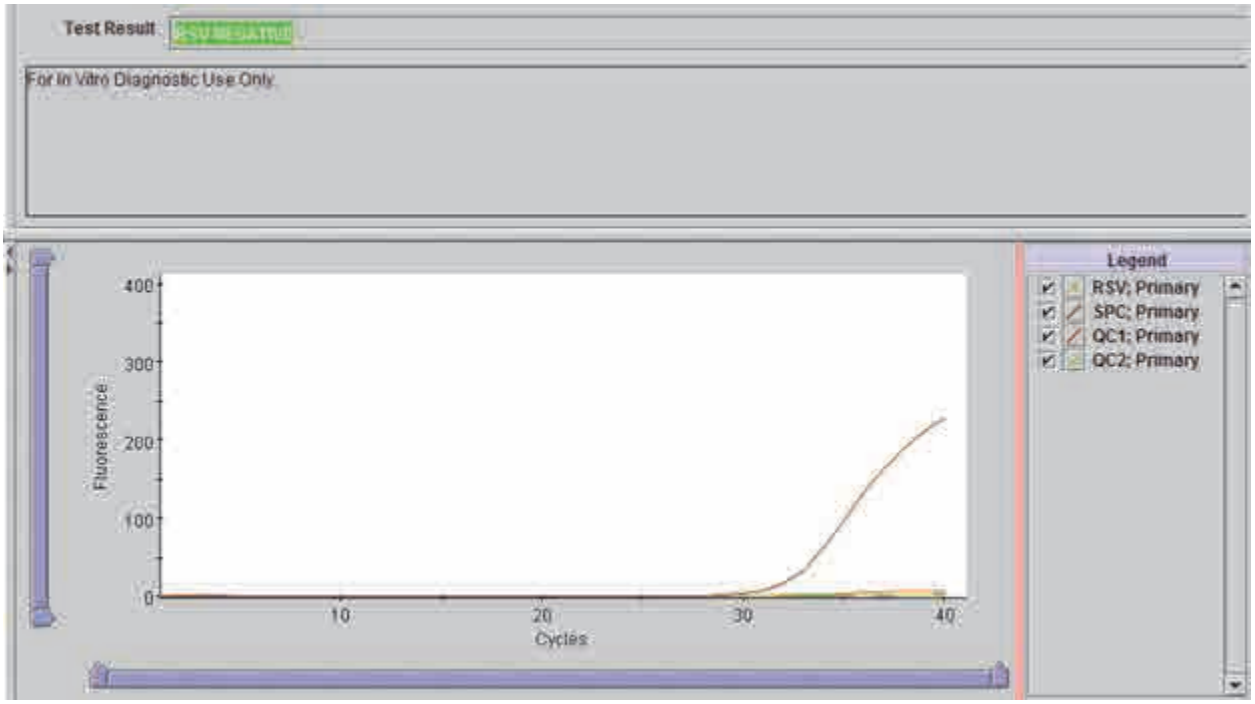

Figure 18. Xpert RSV: An Example of a Negative Result for RSV

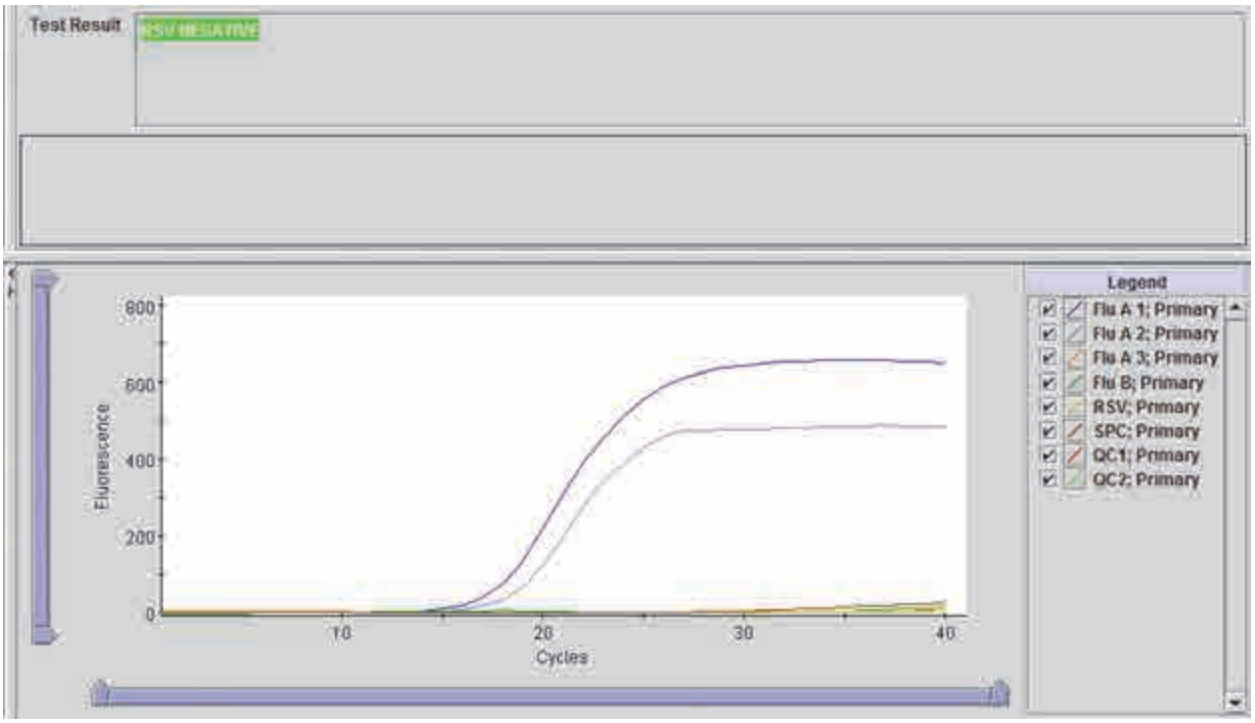

Figure 19. Xpert RSV: An Example of a Negative Result for RSV (Sample Containing Flu A or Flu B Targets)

16 Retests

16.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to the instructions in Section 16.2, Retest Procedure.

- An **INVALID** result indicates that the control SPC failed or that there was amplification for the Flu A 3 target only. The sample was not properly processed, PCR is inhibited or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failed or 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, or a power failure occurred.

16.2 Retest Procedure

For retest of an indeterminate result, use a new cartridge (do not re-use the cartridge).

For NP swab specimens, use 300 µL of the leftover specimen from original transport medium tube.

For NA/W specimens, use 300 µL of the leftover diluted specimen from the 3 mL transport medium tube.

1. Remove a new cartridge from the kit.
2. Mix the specimen by inverting the Xpert Viral Transport Medium tube five times.
3. Open the cartridge lid. Use a clean 300 µL transfer pipette (supplied) to transfer 300 µL (one draw) of the specimen from the transport medium tube to the sample chamber with large opening in the cartridge (Figure 1).
4. Close the cartridge lid.
5. Follow the procedure in Section 12.2, Starting the Test.

17 Limitations

- The performance of the Xpert Flu/RSV XC Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert Flu/RSV XC Assay should be interpreted with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Flu/RSV XC Assay should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.
- If the virus mutates in the target region, influenza virus and RSV may not be detected or may be detected less predictably.
- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2013–2014 influenza season. The performance may vary depending on the prevalence and population tested.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for patients without signs and symptoms of influenza or RSV infection.
- This test has not been evaluated for monitoring treatment of influenza or RSV infection.
- This test has not been evaluated for screening of blood or blood products for the presence of influenza or RSV.

- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- This assay has not been evaluated for immunocompromised individuals.
- Recent patient exposure to FluMist or other live attenuated influenza vaccines may cause inaccurate dual positive results.
- Remel M4 and Remel M4RT transport media are not recommended for use with the Xpert Flu/RSV XC Assay.

18 Performance Characteristics

18.1 Clinical Performance

Performance characteristics of the Xpert Flu/RSV XC Assay were evaluated at six institutions in the U.S. Due to the low prevalence of influenza viruses and the difficulty in obtaining fresh influenza and RSV-positive specimens, the specimen population for this study was supplemented with frozen archived specimens.

Subjects included individuals with signs and symptoms of respiratory infection and whose routine care called for collection of nasal aspirate/wash (NA/W) specimens or nasopharyngeal (NP) swab specimens for influenza and/or RSV testing. For eligible subjects, aliquots of leftover specimens were obtained for testing with the Xpert Flu/RSV XC Assay and reference testing, and patient management continued at the site per their standard practice.

The Xpert Flu/RSV XC Assay performance was compared to a FDA-cleared comparator assay. Bi-directional sequencing was performed on specimens where the Xpert Flu/RSV XC Assay and the comparator assay were discrepant, and is provided for informational purposes only.

18.2 Overall Results

NA/W Specimens

A total of 657 NA/W specimens were tested for influenza A, influenza B and RSV by the Xpert Flu/RSV XC Assay and the reference assay. Of the 657 NA/W specimens, 581 were fresh, prospectively collected and 76 were frozen, archived specimens.

Overall, with NA/W specimens the Xpert Flu/RSV XC Assay demonstrated positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) for detection of influenza A of 98.6%, 100% and 99.8%, respectively relative to the reference assay (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 99.2%, 100%, and 99.8%, respectively (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 97.2%, 99.6%, and 99.1%, respectively (Table 4).

On fresh, prospectively collected NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 100%, 100%, and 100% respectively, relative to the reference assay (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 99.2%, 100%, and 99.8%, respectively (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 98.5%, 99.6%, and 99.3%, respectively (Table 4).

On frozen, archived NA/W specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 97.1%, 100%, and 98.7%, respectively, relative to the reference assay (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 100%, 100%, and 100%, respectively (Table 4). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 84.6%, 100%, and 97.4%, respectively (Table 4).

Table 4. Xpert Flu/RSV XC Assay Performance on NA/W Specimens

Specimen Type	Target	n	TP	FP	TN	FN	PPA % (95 CI)	NPA % (95 CI)	OPA % (95 CI)
Fresh	Flu A	581	35	0	546	0	100 (90.0–100)	100 (99.3–100)	100 (99.4–100)
	Flu B	581	126	0	454	1[a]	99.2 (95.7–100)	100 (99.2–100)	99.8 (99.0–100)
	RSV	581	128	2[b]	449	2[c]	98.5 (94.6–99.8)	99.6 (98.4–99.9)	99.3 (98.2–99.8)
Frozen	Flu A	76	34	0	41	1[d]	97.1 (85.1–99.9)	100 (91.4–100)	98.7 (92.9–100)
	Flu B	76	1	0	75	0	100 (2.5–100)	100 (95.2–100)	100 (95.3–100)
	RSV	76	11	0	63	2[e]	84.6 (54.6–98.1)	100 (94.3–100)	97.4 (90.8–99.7)
All NA/W Specimens	Flu A	657	69	0	587	1[f]	98.6 (92.3–100)	100 (99.4–100)	99.8 (99.2–100)
	Flu B	657	127	0	529	1[g]	99.2 (95.7–100)	100 (99.3–100)	99.8 (99.2–100)
	RSV	657	139	2[h]	512	4[i]	97.2 (93.0–99.2)	99.6 (98.6–100)	99.1 (98.0–99.7)

a Testing results by sequencing: NA; sample not sequenced.
b Testing results by sequencing: 2 of 2 were RSV Positive.
c Testing results by sequencing: 1 of 2 was RSV Positive; 1 of 2 was RSV Negative.
d Testing results by sequencing: 1 of 1 was Flu A Negative.
e Testing results by sequencing: 1 of 2 was RSV Positive; 1 of 2 was RSV Negative.
f Testing results by sequencing: 1 of 1 was Flu A Negative.
g Testing results by sequencing: NA; sample not sequenced.
h Testing results by sequencing: 2 of 2 were RSV Positive.
i Testing results by sequencing: 2 of 4 were RSV Positive; 2 of 4 were RSV Negative.

NP Swab Specimens

A total of 593 NP swab specimens were tested for influenza A, influenza B and RSV by the Xpert Flu/RSV XC Assay and the reference assay. Of the 593 NP swab specimens, 190 were fresh, prospectively collected and 403 were frozen, archived specimens.

Overall, with NP swab specimens the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA and OPA for detection of influenza A of 98.1%, 95.1%, and 95.6%, respectively, relative to the reference assay (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 98.9%, 100%, and 99.8%, respectively (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 91.9%, 99.4%, and 98.7%, respectively (Table 5).

On fresh, prospectively collected NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 85.7%, 98.9%, and 98.4%, respectively, relative to the reference assay (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 100%, 100%, and 100%, respectively (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 100%, 100%, and 100%, respectively (Table 5).

On frozen, archived NP swab specimens, the Xpert Flu/RSV XC Assay demonstrated a PPA, NPA, and OPA for detection of influenza A of 99.0%, 92.8%, and 94.3%, respectively, relative to the reference assay (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for influenza B were 98.8%, 100%, and 99.8%, respectively (Table 5). The Xpert Flu/RSV XC Assay PPA, NPA, and OPA for RSV were 90.4%, 99.1%, and 98.0%, respectively (Table 5).

Table 5. Xpert Flu/RSV XC Assay Performance on NP Swab Specimens

Specimen Type	Target	n	TP	FP	TN	FN	PPA % (95 CI)	NPA % (95 CI)	OPA % (95 CI)
Fresh	Flu A	190	6	2[a]	181	1[b]	85.7 (42.1–99.6)	98.9 (96.1–99.9)	98.4 (95.5–99.7)
	Flu B	190	3	0	187	0	100 (29.2–100)	100 (98.0–100)	100 (98.1–100)
	RSV	190	10	0	180	0	100 (69.2–100)	100 (98.0–100)	100 (98.1–100)
Frozen	Flu A	403	96	22[c]	284	1[d]	99.0 (94.4–100)	92.8 (89.3–95.4)	94.3 (91.6–96.3)
	Flu B	403	85	0	317	1[e]	98.8 (93.7–100)	100 (98.8–100)	99.8 (98.6–100)
	RSV	403	47	3[f]	348	5[g]	90.4 (79.0–96.8)	99.1 (97.5–99.8)	98.0 (96.1–99.1)
All NP Swabs	Flu A	593	102	24[h]	465	2[i]	98.1 (93.2–99.8)	95.1 (92.8–96.8)	95.6 (93.6–97.1)
	Flu B	593	88	0	504	1[j]	98.9 (93.9–100)	100 (99.3–100)	99.8 (99.1–100)
	RSV	593	57	3[k]	528	5[l]	91.9 (82.2–97.3)	99.4 (98.4–99.9)	98.7 (97.4–99.4)

a Testing results by sequencing: 2 of 2 were Flu A Positive.
b Testing results by sequencing: 1 of 1 was Flu A Negative.
c Testing results by sequencing: 17 of 22 were Flu A Positive; 5 of 22 were Flu A Negative.
d Testing results by sequencing: 1 of 1 was Flu A Negative.
e Testing results by sequencing: 1 of 1 was Flu B Negative.
f Testing results by sequencing: 2 of 3 were RSV Positive; 1 of 3 was RSV Negative.
g Testing results by sequencing: 1 of 5 was RSV Positive; 4 of 5 were RSV Negative.
h Testing results by sequencing: 19 of 24 were Flu A Positive; 5 of 24 were Flu A Negative.
i Testing results by sequencing: 2 of 2 were Flu A Negative.
j Testing results by sequencing: 1 of 1 was Flu B Negative.
k Testing results by sequencing: 2 of 3 were RSV Positive; 1 of 3 was RSV Negative.
l Testing results by sequencing: 1 of 5 was RSV Positive; 4 of 5 were RSV Negative.

Of the Xpert Flu/RSV XC Assay runs performed with eligible specimens, 98.6% (1236/1254) of these specimens were successful on the first attempt. The remaining 18 gave indeterminate results on the first attempt (11 **ERROR**, 3 **INVALID** and 4 **NO RESULT**). Seventeen of the 18 specimens were retested, of which 14 yielded valid results after a single retest. There were four NA/W specimens with indeterminate results upon retest which were excluded in the analyses.

19 Analytical Performance

19.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Flu/RSV XC Assay with two lots of reagents across three testing days. The higher LoD observed per strain and per lot was selected for verification. Verification of the estimated LoD claim was performed on one reagent lot across a minimum of three testing days. LoD was established using two influenza A H3N2 strains, two influenza A 2009 H1N1 strains, two influenza B strains, two respiratory syncytial virus A (RSV A) strains, two respiratory syncytial virus B (RSV B) strains, and one influenza A H7N9 strain diluted into a negative pooled clinical matrix. The LoD is defined as the lowest concentration (tissue culture infective dose, $TCID_{50}$/mL) per sample that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which 19 of 20 replicates were positive. Each strain was tested in replicates of 20 per concentration of virus.

The LoD was determined empirically as the first concentration that had 19/20 or 20/20 positive results. The LoD point values for each strain tested are summarized in Table 6 to Table 11.

Table 6. Confirmed LoD ($TCID_{50}$/mL): Influenza A 2009 H1N1

Strain ID	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/California/7/2009	0.3 (20/20)
Influenza A/Florida/27/2011	16 (19/20)

Table 7. Confirmed LoD ($TCID_{50}$/mL): Influenza A H3N2

Strain ID	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/Perth/16/2009	0.3 (20/20)
Influenza A/Victoria/361/2011	0.8 (20/20)

Table 8. Confirmed LoD ($TCID_{50}$/mL): Influenza B

Strain ID	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza B/Massachusetts/2/2012	0.5(20/20)
Influenza B/Wisconsin/01/2010	0.6 (20/20)

Table 9. Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus A

Strain ID	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
RSV A/2/Australia/61	1.2 (20/20)
RSV A/Long/MD/56	1.0 (19/20)

Table 10. Confirmed LoD ($TCID_{50}$/mL): Respiratory Syncytial Virus B

Strain ID	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
RSV B/Washington/18537/62	1.8 (20/20)
RSV B/9320/Massachusetts/77	2.0 (19/20)

Table 11. Confirmed LoD ($TCID_{50}$/mL): Influenza A H7N9

Strain ID	Confirmed LoD ($TCID_{50}$/mL) (at least 19/20 positive)
Influenza A/Anhui/1/2013	21.0 (19/20)

Although this test has been shown to detect the novel avian influenza A(H7N9) cultured material, the performance characteristics of this device with clinical specimens that are positive for the novel avian influenza A(H7N9) virus have not been established. The Xpert Flu/RSV XC Assay can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

19.2 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Flu/RSV XC Assay was evaluated by testing a panel of 44 cultures consisting of 16 viral, 26 bacterial, and two yeast strains representing common respiratory pathogens or those potentially encountered in the nasopharynx. Three replicates of all bacterial and yeast strains were tested at concentrations of $\geq 10^6$ CFU/mL with the exception of one strain which was tested at 10^5 CFU/mL (*Chlamydia pneumoniae*). Three replicates of all viruses were tested at concentrations of $\geq 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 12.

Table 12. Analytical Specificity of Xpert Flu/RSV XC Assay

Organism	Concentration	Result		
		Flu A	Flu B	RSV
No Template Control	N/A	NEG	NEG	NEG
Adenovirus Type 1	1.12×10^7 $TCID_{50}$/mL	NEG	NEG	NEG
Adenovirus Type 7	1.87×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus OC43	2.85×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus 229E	1×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Cytomegalovirus	7.24×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Echovirus	3.31×10^7 $TCID_{50}$/mL	NEG	NEG	NEG
Enterovirus	1×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Epstein Barr Virus	7.16×10^7 $TCID_{50}$/mL	NEG	NEG	NEG
HSV	8.9×10^6 $TCID_{50}$/mL	NEG	NEG	NEG
Measles	6.3×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Human metapneumovirus	3.8×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Mumps virus	6.31×10^6 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 1	1.15×10^6 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 2	1×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza Type 3	3.55×10^7 $TCID_{50}$/mL	NEG	NEG	NEG
Rhinovirus Type 1A	1.26×10^5 $TCID_{50}$/mL	NEG	NEG	NEG
Acinetobacter baumannii	$> 1 \times 10^6$ CFU/mL	NEG[a]	NEG	NEG
Burkholderia cepacia	$> 1 \times 10^6$ CFU/mL	NEG	NEG	NEG
Candida albicans	$> 1 \times 10^6$ CFU/mL	NEG	NEG	NEG
Candida parapsilosis	$> 1 \times 10^6$ CFU/mL	NEG	NEG	NEG
Bordetella pertussis	1×10^8 CFU/mL	NEG	NEG	NEG
Chlamydia pneumoniae	3.16×10^5 CFU/mL	NEG	NEG	NEG
Citrobacter freundii	$> 1 \times 10^6$ CFU/mL	NEG	NEG	NEG
Corynebacterium sp.	$> 1 \times 10^6$ CFU/mL	NEG	NEG	NEG
Escherichia coli	$> 1 \times 10^6$ CFU/mL	NEG	NEG	NEG

Table 12. Analytical Specificity of Xpert Flu/RSV XC Assay (Continued)

Organism	Concentration	Result		
		Flu A	Flu B	RSV
Enterococcus faecalis	> 1×10^6 CFU/mL	NEG	NEG	NEG
Hemophilus influenzae	1×10^6 CFU/mL	NEG	NEG	NEG
Lactobacillus sp.	1×10^6 CFU/mL	NEG	NEG	NEG
Legionella spp.	1×10^8 CFU/mL	NEG	NEG	NEG
Moraxella catarrhalis	> 1×10^6 CFU/mL	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.15×10^6 CFU/mL	NEG	NEG	NEG
Mycoplasma pneumoniae	1×10^7 CFU/mL	NEG	NEG	NEG
Neisseria meningitidis	> 1×10^6 CFU/mL	NEG	NEG	NEG
Neisseria mucosa	> 1×10^6 CFU/mL	NEG	NEG	NEG
Propionibacterium acnes	> 1×10^6 CFU/mL	NEG	NEG	NEG
Pseudomonas aeruginosa	> 1×10^6 CFU/mL	NEG	NEG	NEG
Staphylococcus aureus	> 1×10^6 CFU/mL	NEG	NEG	NEG
Staphylococcus epidermidis	> 1×10^6 CFU/mL	NEG	NEG	NEG
Staphylococcus haemolyticus	> 1×10^6 CFU/mL	NEG	NEG	NEG
Streptococcus agalactiae	> 1×10^6 CFU/mL	NEG	NEG	NEG
Streptococcus pneumoniae	> 1×10^6 CFU/mL	NEG	NEG	NEG
Streptococcus pyogenes	> 1×10^6 CFU/mL	NEG	NEG	NEG
Streptococcus salivarius	> 1×10^6 CFU/mL	NEG	NEG	NEG
Streptococcus sanguinis	> 1×10^6 CFU/mL	NEG	NEG	NEG

a For *Acinetobacter baumannii* upon initial testing 1 of 3 replicates was positive for Flu A with a Ct of 39.2 (cut-off = 40). An additional 23 replicates were tested at > 1×10^6 CFU/mL; 23 of 23 replicates were correctly reported as **Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE**.

19.3 Analytical Reactivity (Inclusivity)

The analytical reactivity of the Xpert Flu/RSV XC Assay was evaluated against multiple strains of influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 64 strains including 54 influenza viruses and 10 RSV strains were tested in this study with the Xpert Flu/RSV XC Assay.

Three replicates were tested for each strain. Results are shown in Table 13.

Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
No Template Control		N/A	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/WS/33	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/PR/8/34	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Mal/302/54	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Denver/1/57	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Jersey/8/76	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Caledonia/20/1999	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/New York/55/2004	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Solomon Island/3/2006	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Taiwan/42/06	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/59/2007	32.0 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/California/7/2009	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/swine/NY/02/2009	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Florida/27/2011	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Colorado/14/2012	32.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Washington/24/2012	80.0[a] $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hong Kong/8/68	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Port Chalmers/1/73	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hawaii/15/2001	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Wisconsin/67/05	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/10/2007	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Perth/16/2009	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Minnesota/11/2010 (H3N2)v	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Indiana/08/2011 (H3N2)v	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Victoria/361/2011	1.6 $TCID_{50}$/mL	POS	NEG	NEG
	A/Texas/50/2012	1.6 $TCID_{50}$/mL	POS	NEG	NEG

Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay (Continued)

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
Avian influenza A	A/duck/Hunan/795/2002 (H5N1)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/chicken/Hubei/327/2004 (H5N1)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/ 1038/2006 (H5N1)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/duck/LTC-10-82743/1943 (H7N2)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/chicken/NJ/15086-3/94 (H7N3)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	N/A[c]	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	N/A[c]	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/ 1996 (H9N2)	≤ 1pg/µL[b]	POS	NEG	NEG
	A/Mallard/NY/6750/78 (H2N2)	≤ 1pg/µL[b]	POS	NEG	NEG
Influenza B	B/Lee/40	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Allen/45	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/GL/1739/54	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Maryland/1/59	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Panama/45/90[d]	3.0 $TCID_{50}$/mL[e]	NEG	POS	NEG
	B/Florida/07/2004[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/02/06[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/04/06[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Wisconsin/01/2011[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Massachusetts/2/2012[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Hong Kong/5/72	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Wisconsin/01/2010[f]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Malaysia/2506/04[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Taiwan/2/62	1.2 $TCID_{50}$/mL	NEG	POS	NEG
	B/Brisbane/60/2008[d]	1.2 $TCID_{50}$/mL	NEG	POS	NEG
RSV A	RSV-A/Long/MD/56	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/2/Australia/61	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/NY (Clinical unknown)	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-8-2/2007	2.4 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-11-1/2008	2.4 $TCID_{50}$/mL	NEG	NEG	POS

Table 13. Analytical Reactivity (Inclusivity) of Xpert Flu/RSV XC Assay (Continued)

Virus	Strain	Concentration	Result		
			Flu A	Flu B	RSV
RSV B	RSV-B/Wash/18537/62	4.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/9320/MA/77	4.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/WV14617/85	4.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/CH93(18)-18	20.0 $TCID_{50}$/mL[g]	NEG	NEG	POS
	RSV-B/WI/629-5B/0607	4.0 $TCID_{50}$/mL	NEG	NEG	POS

a Influenza A/Washington/24/2012 was tested at 5X LoD (80.0 $TCID_{50}$/mL) to obtain 3 of 3 **Flu A POSITIVE** result calls.
b Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.
c Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000 fold in simulated background matrix and tested due to biosafety regulations.
d Known Victoria lineage.
e Influenza B/Panama/45/90 was tested at 5X LoD (3.0 $TCID_{50}$/mL) to obtain 3 of 3 **Flu B POSITIVE** result calls.
f Known Yamagata lineage.
g RSV-B/CH93(18)-18 was tested at 10X LoD (20.0 $TCID_{50}$/mL) to obtain 3 of 3 **RSV POSITIVE** result calls.

19.4 Interfering Substances Study

In a non-clinical study, potentially interfering substances that may be present in the nasopharynx were evaluated directly relative to the performance of the Xpert Flu/RSV XC Assay. Potentially interfering substances in the nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Negative samples (n = 8) were tested per each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n = 8) were tested per substance with six influenza (four influenza A and two influenza B) and four RSV (two RSV A and two RSV B) strains spiked at 2X the analytical LoD determined for each strain. All results were compared to positive and negative Universal Transport Medium (UTM) controls.

These evaluated substances are listed in Table 14 with active ingredients and concentrations tested shown. There was no assay interference in the presence of the substances at the concentrations tested in this study. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

FluMist vaccine samples were correctly reported as **Flu A POSITIVE**; **FLU B POSITIVE**; **RSV NEGATIVE** as expected. Samples containing FluMist may cause false positive results. This is addressed in Section 17, Limitations.

Table 14. Potentially Interfering Substances in Xpert Flu/RSV XC Assay

Substance/Class	Description/Active Ingredient	Concentration Tested
Beta-adrenergic bronchodilator	Albuterol Sulfate	0.83 mg/mL (equivalent to 1 dose per day)
Blood	Blood (Human)	2% (v/v)
BD™ Universal Viral Transport System	Transport Media	100% (v/v)
Remel M4®	Transport Media	100% (v/v)
Remel M4RT®	Transport Media	100% (v/v)
Remel M5®	Transport Media	100% (v/v)
Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	1.7 mg/mL
Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)	2.5% (w/v)
Antibiotic, nasal ointment	Mupirocin	10 mg/mL
Saline Nasal Spray	Sodium Chloride (0.65%)	15% (v/v)

Table 14. Potentially Interfering Substances in Xpert Flu/RSV XC Assay (Continued)

Substance/Class	Description/Active Ingredient	Concentration Tested
Anefrin Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
Nasal Drops	Phenylephrine, 0.5%	15% (v/v)
Tamiflu®/Anti-viral drugs	Zanamivir	7.5 mg/mL
Antibacterial systemic	Tobramycin	4 µg/mL
Zicam®/Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum Hydrochloricum Sulfur	15% (w/v)
FluMist®	Live intranasal influenza virus vaccine	6.7% (v/v)
Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

19.5 Carry-over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples run followed by very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately followed by a very high influenza A sample (approximately 10^6 $TCID_{50}$/test) or a very high RSV A sample (approximately 10^6 $TCID_{50}$/test). This testing scheme was repeated 20 times on two GeneXpert modules for a total of 82 runs resulting in 40 positive and 42 negative specimens for each virus type. All 40 positive samples were correctly reported as **Flu A POSITIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE** or **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV POSITIVE**. All 42 negative samples were correctly reported as **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE**.

19.6 Fresh vs Frozen Sample Equivalency Study

Fresh and frozen specimen equivalency in the Xpert Flu/RSV XC Assay was evaluated by testing individual influenza and RSV strains at three different concentrations representing low positives (2X LoD), moderate positives (5X LoD), and high positives (10X LoD) in simulated background matrix. Negative samples consisted of simulated background matrix only. Fresh and frozen specimen equivalency was determined using one seasonal Flu A H3N2 strain (A/Victoria/361/2011), one Flu B strain (B/Wisconsin/01/11), one RSV A strain (RSV A/Long/MD/56), and one RSV B strain (RSV B/9320/MA/77). Replicates of 20 were tested for each specimen type and concentration. All positive and negative specimens were tested fresh, after one freeze-thaw cycle, and after two freeze-thaw cycles.

There was no statistically significant effect in the performance of the Xpert Flu/RSV XC Assay between fresh virus dilutions and two sequential freeze thaw cycles for positive and negative samples. All positive and negative replicates were correctly identified using the Xpert Flu/RSV XC Assay.

20 Reproducibility

A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on ten different days by two different operators, at each of three sites (10 specimens × 1 time/day × 10 days × 2 operators × 3 sites). One lot of Xpert Flu/RSV XC Assay cartridges was used at each of the 3 testing sites. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 15.

Table 15. Summary of Reproducibility Results

Sample ID	Site 1/GX Dx			Site 2/Infinity-80			Site 3/Infinity-48			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (60/60)
Flu A - High Neg	70.0% (7/10)	60.0% (6/10)	65.0% (13/20)	80.0% (8/10)	80.0% (8/10)	80.0% (16/20)	60.0% (6/10)	70.0% (7/10)	65.0% (13/20)	70.0% (42/60)
Flu A - Low Pos	100% (10/10)	90.0% (9/10)	95.0% (19/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	90.0% (9/10)	95.0% (19/20)	96.7% (58/60)
Flu A - Mod Pos	100% (10/10)	90.0% (9/10)	95.0% (19/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	98.3% (59/60)
Flu B - High Neg	90.0% (9/10)	70.0% (7/10)	80.0% (16/20)	100% (10/10)	70.0% (7/10)	85.0% (17/20)	50.0% (5/10)	80.0% (8/10)	65.0% (13/20)	76.7% (46/60)
Flu B - Low Pos	100% (10/10)	90.0% (9/10)	95.0% (19/20)	90.0% (9/10)	70.0% (7/10)	80.0% (16/20)	100% (10/10)	90.0% (9/10)	95.0% (19/20)	90.0% (54/60)
Flu B - Mod Pos	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (60/60)
RSV- High Neg	60.0% (6/10)	50.0% (5/10)	55.0% (11/20)	90.0% (9/10)	60.0% (6/10)	75.0% (15/20)	70.0% (7/10)	70.0% (7/10)	70.0% (14/20)	66.7% (40/60)
RSV - Low Pos	77.8%[a] (7/9)	100% (10/10)	89.5% (17/19)	80.0% (8/10)	80.0% (8/10)	80.0% (16/20)	90.0% (9/10)	90.0% (9/10)	90.0% (18/20)	86.4% (51/59)
RSV - Mod Pos	100%[b] (9/9)	100% (10/10)	100% (19/19)	100% (10/10)	100% (10/10)	100% (20/20)	100% (10/10)	100% (10/10)	100% (20/20)	100% (59/59)

a One sample indeterminate on initial testing; retest not done.
b One sample 2x indeterminate.

The reproducibility of the Xpert Flu/RSV XC Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-days and between-operators for each panel member are presented in Table 16. One replicate was performed per day per operator; therefore, operator and assay (within-run) precision are confounded.

Table 16. Summary of Reproducibility Data

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Site		Between-Day		Between-Operator + Within Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	60	30.8	0.06	0.2	0	0	0.29	0.9	0.29	0.9
Flu A - High Neg	FluA1	18	38.0	0	0	1.55	4.1	0.85	2.2	1.77	4.6
	FluA2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A - Low Pos	FluA1	58	34.9	0.38	1.1	0.10	0.3	1.28	3.7	1.34	3.8
	FluA2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A - Mod Pos	FluA1	59	33.5	0.49	1.5	0	0	1.29	3.9	1.38	4.1
	FluA2	10	36.3	NA	NA	NA	NA	NA	NA	NA	NA
	FluA3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu B - High Neg	FluB	14	36.6	0.80	1.4	0	0	2.83	7.7	2.94	8.0
Flu B - Low Pos	FluB	54	33.4	0	0	1.07	3.2	1.76	5.3	2.06	6.2
Flu B - Mod Pos	FluB	60	32.1	0	0	0.38	1.2	1.47	4.6	1.51	4.7
RSV - High Neg	RSV	20	37.4	0	0	0.14	0.4	1.68	4.5	1.68	4.5
RSV - Low Pos	RSV	51	36.2	0.22	0.6	0	0	1.75	4.8	1.76	4.9
RSV - Mod Pos	RSV	60	35.1	0	0	0.24	0.9	1.20	3.4	1.24	3.5

a Results with non-zero Ct values out of 60.

21 Instrument System Precision

An in-house precision study was conducted to compare the performance of the GeneXpert Dx and the GeneXpert Infinity instrument systems. A panel of 10 specimens with varying concentrations of influenza A, influenza B, and RSV was tested on 12 different days by two operators. Each operator conducted four runs of each panel specimen per day on each of the two instrument systems (10 specimens × 2 times/ day × 12 days × 2 operators × 2 instrument systems). Three lots of Xpert Flu/RSV XC Assay cartridges were used for the study. The Xpert Flu/RSV XC Assay was performed according to the Xpert Flu/RSV XC Assay procedure. Results are summarized in Table 17.

Table 17. Summary of Instrument System Precision Results (Dx vs. Infinity)

Sample	GeneXpert Dx			Infinity			% Total Agreement by Sample
	Op 1	Op 2	Inst	Op 1	Op 2	Inst	
Negative	100% (48/48)	100% (48/48)	100% (96/96)	100% (48/48)	100% (48/48)	100% (96/96)	100% (192/192)
Flu A - High Neg	75.0% (36/48)	77.1% (37/48)	76.0% (73/96)	87.5% (42/48)	75.0% (36/48)	81.3% (78/96)	78.7% (151/192)
Flu A - Low Pos	68.8% (33/48)	97.9% (47/48)	83.3% (80/96)	91.7% (44/48)	93.8% (45/48)	92.7% (89/96)	88.0% (169/192)
Flu A - Mod Pos	97.9% (47/48)	100% (48/48)	99.0% (95/96)	93.8% (45/48)	97.9% (47/48)	95.8% (92/96)	97.4% (187/192)
Flu B - High Neg	81.3% (39/48)	79.2% (38/48)	80.2% (77/96)	89.6% (43/48)	79.2% (38/48)	84.4% (81/96)	82.3% (158/192)

Table 17. Summary of Instrument System Precision Results (Dx vs. Infinity) (Continued)

Sample	GeneXpert Dx			Infinity			% Total Agreement by Sample
	Op 1	Op 2	Inst	Op 1	Op 2	Inst	
Flu B - Low Pos	89.6% (43/48)	95.8% (46/48)	92.7% (89/96)	89.6% (43/48)	87.5% (42/48)	88.5% (85/96)	90.6% (174/192)
Flu B - Mod Pos	97.9% (47/48)	100% (48/48)	99.0% (95/96)	100% (48/48)	100% (48/48)	100% (96/96)	99.5% (191/192)
RSV- High Neg	89.6% (43/48)	77.1% (37/48)	83.3% (80/96)	87.5% (42/48)	83.3% (40/48)	85.4% (82/96)	84.4% (162/192)
RSV - Low Pos	93.8% (45/48)	93.8% (45/48)	93.8% (90/96)	87.5% (42/48)	89.6% (43/48)	88.5% (85/96)	91.1% (175/192)
RSV - Mod Pos	100% (48/48)	100% (48/48)	100% (96/96)	97.9% (47/48)	100% (48/48)	99.0% (95/96)	99.5% (191/192)

The precision of the Xpert Flu/RSV XC Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-instruments, between-lots, between-days, between-operators, and within-assays for each panel member are presented in Table 18.

Table 18. Summary of Precision Data

Sample	Assay Channel (Analyte)	N[a]	Mean Ct	Between-Instrument		Between-Lot		Between-Day		Between-Operator		Within-Assay		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	SPC	192	30.6	0	0	0.19	0.6	0.06	0.2	0.02	0.1	0.36	1.2	0.41	1.3
Flu A - High Neg	FluA1	41	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A - Low Pos	FluA1	169	35.6	0	0	0.42	1.2	0.93	2.6	0.28	0.8	1.61	4.5	1.93	5.4
	FluA2	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A - Mod Pos	FluA1	187	34.1	0	0	0.41	1.2	0.95	2.8	0	0	1.54	4.5	1.86	5.5
	FluA2	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	FluA3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu B- High Neg	FluB	34	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Flu A - Low Pos	FluB	174	33.2	0	0	0.47	1.4	0	0	0.66	2.0	2.03	6.1	2.18	6.6
Flu A - Mod Pos	FluB	191	32.1	0	0	0.17	0.5	0.25	0.8	0	0	1.73	5.4	1.75	5.5
RSV - High Neg	RSV	30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RSV - Low Pos	RSV	175	36.0	0	0	0.75	2.1	0	0	0.36	1.0	1.47	4.1	1.69	4.7
RSV - Mod Pos	RSV	191	34.7	0	0	0.57	1.7	0.16	0.5	0	0	1.23	3.6	1.37	3.9

a Results with non-zero Ct values out of 192.

22 References

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23 Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408.541.4191	Telephone: +33 563 825 300
Fax: +1 408.541.4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com/

24 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

Region	Telephone	Email
US	+1 888.838.3222	TechSupport@cepheid.com
France	+33 563 825 319	Support@cepheideurope.com
Germany	+49 69 710 480 480	Support@cepheideurope.com
United Kingdom	+44 3303 332 533	Support@cepheideurope.com
South Africa	+27 87 808 1600	Support@cepheideurope.com
Other European, Middle East and African countries	+33 563 825 319 +971 4 253 3218	Support@cepheideurope.com
Australia, New Zealand	+61 1800 107 884	Support@cepheideurope.com
Other countries not listed above	+1 408.400.8495	TechSupport@cepheid.com

Contact information for other Cepheid offices is available on our website at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab. Select the **Contact Us** option.

25 Table of Symbols

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

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA
Phone: +1.408.541.4191
Fax: +1.408.541.4192
www.cepheid.com

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France
Phone: +33 563 825 300
Fax: +33 563 825 301
www.cepheidinternational.com/

IVD CE

Dalinis dokumento vertimas iš anglų kalbos

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Flu/RSV XC

REF **GXFLU/RSV-CE-10**

Cepheid

In Vitro diagnostinė medicinos priemonė

CE IVD

301-2904, Peržiūra B, 2015 m. rugsėjo mėn.

Tik *In Vitro* diagnostiniam naudojimui.

Patentuotas pavadinimas
Xpert® Flu/RSV XC

Bendrinis / įprastinis pavadinimas
Xpert Flu/RSV XC tyrimas

Paskirtis
Cepheid® Xpert Flu/RSV XC tyrimas yra automatizuotas, sudėtinis tikro laiko, atvirkštinės transkriptazės polimerazės grandinės reakcijos (RT-PGR) tyrimas, skirtas in vitro kokybiniam *Influenza A*, *Influenza B* ir respiratorinio sincitinio viruso (RSV) RNR nustatymui ir diferenciacijai. Xpert Flu/RSV XC tyrime yra naudojami nosies aspirato/nuoplovų ir nosiaryklės tepinėlių mėginiai, surinkti iš pacientų, turinčių kvėpavimo takų infekcijos požymius. Xpert Flu/RSV XC tyrimas yra naudojamas kaip pagalbinė priemonė atliekant gripo ir respiratorinio sincitinio viruso infekcijos diagnozę kartu su klinikiniais bei epidemiologiniais rizikos faktoriais.

Procedūros principas
Xpert Flu/RSV XC tyrimas yra greitas, automatizuotas in vitro diagnostinis testas, skirtas kokybiniam *Influenza A*, *Influenza B* ir RSV nustatymui. Tyrimas yra atliekamas su Cepheid GeneXpert instrumentų sistemomis.
GeneXpert instrumentų sistemos integruoja ir automatizuoja mėginio ekstrakciją, nukleininės rūgšties išgryninimą, amplifikaciją bei taikinio eilių aptikimą klinikiniuose mėginiuose, naudojant atvirkštinę transkripciją (RNR šablonų konversija į DNR) ir tikro laiko PGR. Sistemą sudaro instrumentas, personalinis kompiuteris ir įdiegta programinė įranga, skirta mėginių tyrimų paleidimui ir rezultatų peržiūrai. Sistemai yra reikalingos vienkartinio naudojimo kasetės, kuriose yra taikiniui specifiški reagentai ir kuriose vyksta RT-PGR ir PGR procesai. Dėl pilno sistemos aprašymo prašome žiūrėti *GeneXpert Dx System* arba *GeneXpert Infinity System* naudotojo vadovą.

Xpert Flu/RSV XC tyrimo sudėtyje yra reagentų, skirtų *Influenza A*, *Influenza B* ir RSV aptikimui ir diferenciacijai tiesiogiai iš nosies aspiratų/nuoplovų (NA/W) bei nosiaryklės (NP)tepinėlių mėginių, surinktų iš pacientų, kuriems yra įtariama kvėpavimo takų infekcija. Kasetėje yra mėginio apdorojimo kontrolė (SPC) ir tyrimo patikros kontrolė (PCC). Mėginio apdorojimo kontrolė (SPC) yra skirta adekvataus taikinio viruso apdorojimo atlikimui ir inhibitorių stebėjimui PGR reakcijoje. Tyrimo tikrinimo kontrolė (PCC) patikrina reagento rehidraciją, PGR mėgintuvėlio užpildymą kasetėje, mėgintuvėlio integralumą ir dažų stabilumą.

Laikymas ir naudojimas

- Xpert Flu/RSV XC tyrimo kasetės ir reagentai turi būti laikomi prie 2–28 °C iki galiojimo datos pabaigos, nurodytos pakuotės etiketėje.
- Neatidarykite kasetės tol, kol nebūsite pasiruošę atlikti tyrimo.
- Nenaudokite pasibaigusio galiojimo kasečių.
- Nenaudokite kasetės, kurios turinys yra pratekėjęs.

Įspėjimai ir atsargumo priemonės

•Tik *in vitro* diagnostiniam naudojimui.

•Su visais biologiniais mėginiais, įskaitant panaudotas kasetes, elkitės kaip su galinčiais pernešti infekcinius agentus. Kadangi nėra žinoma, kuris mėginys yra infekciškas, su visais biologiniais mėginiais reikia dirbti laikantis universaliųjų atsargumo priemonių.

• Jei, remiantis esamais klinikiniais ir epidemiologiniais skryningo kriterijais, yra įtariamas naujas *Influenza A* virusas, mėginiai turi būti surenkami laikantis atitinkamų infekcijos kontrolės atsargumo priemonių ir siunčiami į valstybinį ar vietinį sveikatos departamentą ištyrimui. Tokiu atveju virusinė kultūra neturi būti išgaunama, nebent yra galimybė mėginius kultivuoti BSL 3+ įstaigoje.

• Šio tyrimo veiksmingumo charakteristika buvo nustatoma su mėginių tipais, nurdytais paskirties skyriuje. Šio tyrimo veiksmingumas su kito tipo mėginiais nebuvo nustatomas.

• Dėl darbo su chemikalais ir biologiniais mėginiais, laikykitės Jūsų laboratorijoje atliekamų saugos procedūrų.

• Dėl tinkamo kasečių ir nepanaudotų reagentų išmetimo pasitarkite su savo įstaigos atliekų utilizavimo skyriumi. Ši medžiaga gali turėti valstybinio EPA išteklių saugojimo ir atstatymo akto (RCRA) charakteristiką dėl pavojingų atliekų, kurioms yra taikomi specifiniai utilizavimo reikalavimai. Įstaigos turi laikytis savo šalyje galiojančių nurodymų dėl pavojingų atliekų utilizavimo.

• Lizuojančio reagento sudėtyje yra guanidinio tiocianato (H302, H316, H320, H402, EUH031), kuris yra pavojingas vandens gyvūnijai ir įvykus kontaktui su rūgštimis, skleidžia toksiškas dujas.

Techninė pagalba

Skambinant ar rašant el.laišką į Cepheid techninės pagalbos skyrių, turėkite šią informaciją:

• Produkto pavadinimas
• Serijos numeris
• Instrumento serijos numeris
• Klaidų pranešimai (jei yra)
• Programinės įrangos versija ir, jei taikoma, kompiuterio serverio numeris.

Regionas	Telefonas	El. paštas
JAV	+1 888.838.3222	TechSupport@cepheid.com
Prancūzija	+33 563 825 319	Support@cepheideurope.com
Vokietija	+49 69 710 480 480	Support@cepheideurope.com
Jungtinė Karalystė	+44 3303 332 533	Support@cepheideurope.com
Pietų Afrika	+27 87 808 1600	Support@cepheideurope.com
Kitos Europos, Vidurio Rytų ir Afrikos šalys	+33 563 825 319 +971 4 253 3218	Support@cepheideurope.com
Australija, Naujoji Zelandija	+61 1800 107 884	Support@cepheideurope.com
Kitos šalys	+1 408.400.8495	TechSupport@cepheid.com

Gamintojas
Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089-1189
USA
Tel.: +1.408.541.4191
Faks.: +1.408.541.4192

Įgaliotas atstovas
Cepheid Europe
Vira Solelh
81470 Maurens-Scopont
France
Tel: +33.563.82.53.00
Fax: +33.563.82.53.01
Email: cepheid@cepheideurope.fr

Tikslus dokumento vertimas į lietuvių kalb
Vertėjas(-a) A. Gegeckienė
Data: 2016-10-3
UAB Diamedica
Molėtų pl. 73, Vilnius
Lietuva
Tel. 8 5 279 0080

GeneXpert®
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Xpert® MTB/RIF Ultra

REF **GXMTB/RIF-ULTRA-10**
GXMTB/RIF-ULTRA-50

Cepheid® *In Vitro* Diagnostic Medical Device IVD CE

301-5987, Rev. D May 2017

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Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

Xpert® MTB/RIF Ultra

For *In Vitro* Diagnostic Use

Proprietary Name

Xpert® MTB/RIF Ultra

Common or Usual Name

Xpert MTB/RIF Ultra Assay

A. Intended Use

The Xpert MTB/RIF Ultra Assay, performed on the GeneXpert Instrument Systems, is a semi-quantitative, nested real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for the detection of *Mycobacterium tuberculosis* (MTB) complex DNA in unprocessed sputum samples or concentrated sediments prepared from induced or expectorated sputum. In specimens where *Mycobacterium tuberculosis* complex is detected, the Xpert MTB/RIF Ultra Assay can also detect rifampin-resistance associated mutations of the *rpoB* gene.

The Xpert MTB/RIF Ultra Assay is intended for use with specimens from patients for whom there is clinical suspicion of tuberculosis (TB) and who have received no antituberculosis therapy, or less than 3 days of therapy in the last 6 months. This test is intended as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings.

B. Summary and Explanation

Globally, about 2 billion people are infected with MTB.[1] In 2015, 10.4 million people developed active disease, and 1.4 million people lost their lives to the illness.[2] The route of transmission of pulmonary TB is through the air, which makes this a highly transmissible disease. Given the infectious nature of pulmonary TB, fast and accurate diagnosis is an important element of TB treatment and control.

Treatment involves prolonged administration of multiple drugs and is usually highly effective. However, *M. tuberculosis* strains may become resistant to one or more of the drugs, making cure much more difficult to achieve. Four common first-line drugs used in anti-tuberculosis therapy are isoniazid (INH), rifampin (also known as rifampicin, RIF), ethambutol (EMB), and pyrazinamide (PZA). As documented by World Health Organization, RIF resistance is rarely encountered by itself, and usually indicates resistance to a number of other anti-TB drugs.[3] It is most commonly seen in multi-drug resistant (MDR-TB) strains (defined as resistant to both RIF and INH) and has a reported frequency of greater than 95% in such isolates.[4,5,6] Resistance to RIF or other first-line drugs usually indicates the need for full susceptibility testing, including testing against second-line agents.

Molecular detection of TB and *rpoB* gene mutations associated with RIF resistance greatly reduces the time to diagnosis of both drug-susceptible and MDR tuberculosis. With the Xpert MTB/RIF Ultra Assay, this can be accomplished in unprocessed sputum samples and in prepared sediments in less than 80 minutes. The rapid detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy during a single medical encounter.

C. Principle of the Procedure

The GeneXpert Instrument Systems integrate and automate sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and melt peak detection. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on patient samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, 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 MTB/RIF Ultra Assay includes reagents for the detection of MTB and RIF resistance and a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor for the presence of inhibitor(s) in the PCR reaction and subsequent melt peak detection. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers in the Xpert MTB/RIF Ultra Assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region and portions of the multi-copy *IS1081* and *IS6110* insertion elements target sequences. The melt analysis with four *rpoB* probes is able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance. The two insertion element probes enhance the detection of *Mycobacterium tuberculosis* complex due to the multi-copy insertion element target sequences in most TB strains.

D. Reagents and Instruments

D.1 Materials Provided

The Xpert MTB/RIF Ultra Assay kits contain sufficient reagents to process 10 samples or 50 samples. The kits contain the following:

Xpert MTB/RIF Ultra Assay Cartridges with Integrated Reaction Tubes	**10 per kit**	**50 per kit**
• Bead 1 and Bead 2 (freeze-dried)	2 of each per cartridge	2 of each per cartridge
• Bead 3 (freeze-dried)	1 of each per cartridge	1 of each per cartridge
• Reagent 1	4 mL per cartridge	4 mL per cartridge
• Reagent 2	4 mL per cartridge	4 mL per cartridge
Sample Reagent Bottles	**10**	**50**
• Sample Reagent	8 mL per bottle	8 mL per bottle
Disposable Transfer Pipettes	**12 per kit**	**60 per kit**
CD	**1**	**1**
• Assay Definition Files (ADF)		
• Instructions to import ADF into software		
• Package Insert		

Note Sample Reagent (SR) can be colorless to yellow to amber. Color may intensify with time, but color has no effect on performance.

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

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

Note The transfer pipettes have a single mark representing the minimum volume of treated sample necessary to transfer to the cartridge. Use only for this purpose. All other pipettes must be provided by the laboratory.

D.2 Storage and Handling

- Store the Xpert MTB/RIF Ultra Assay cartridges at 2–28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use reagents or cartridges that have passed the expiration date.

E. Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert Instrument, computer, barcode scanner, and operator manual
  - For GeneXpert Dx system: Software version 4.7b or higher
  - For GeneXpert Infinity system: Software version 6.4b or higher
- Printer: If a printer is required, contact Cepheid Sales Representative to arrange for the purchase of a recommended printer.
- Leak-proof, sterile screw-capped collection containers
- Disposable gloves
- Labels and/or indelible labeling marker
- Sterile pipettes for sample processing

F. Warnings, Precautions and Chemical Hazards

F.1 Warnings and Precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute.[8]
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly after handling samples and test reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Xpert MTB/RIF Ultra Assay reagents with other reagents.
- Do not open the Xpert MTB/RIF Ultra Assay cartridge lid except when adding treated sample.
- Do not use a cartridge that has been dropped after removing from the kit.
- Do not use a cartridge that has been dropped or shaken or has spilled contents of cartridge after you have added the treated sample. Shaking or dropping the cartridge after opening the lid may yield false or non-determinate results.
- Do not place the Sample ID label on the cartridge lid or on the bar code label.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use a cartridge that has a damaged reaction tube.
- When processing more than one sample at a time, open only one cartridge; add the Sample Reagent-treated sample and close the cartridge lid before processing the next sample. Change gloves between samples.

- Each Xpert MTB/RIF Ultra Assay cartridge is used to process one test. Do not reuse processed cartridges.
- Good laboratory practices should be followed and gloves should be changed between handling each patient specimen in order to avoid contamination of specimens or reagents. Regularly clean the work surface/areas with 10% bleach then wipe the surface again with 70% ethanol or isopropyl alcohol before and after processing specimens.
- Check your regional/country hazardous and medical waste disposal requirements. If regulations do not provide clear directions on the proper disposal of specimens or used cartridges, they should be treated as capable of transmitting infectious agents. Dispose of the used cartridges as chemical hazardous health-care waste in durable waste containers per WHO (World Health Organization) following medical waste handling and disposal guidelines.

F.2 Chemical Hazards[9,10]

Sample Reagent:

- CLP Hazard Pictogram:

- Signal Word: DANGER
- Hazard Class: Flammable Liquids 3, Skin Corrosion 1A
- Contains Isopropyl Alcohol
  - Hazard Statement: H226: Flammable liquid and vapor
- Contains Sodium Hydroxide
  - Hazard Statement: H314: Causes severe skin burns and eye damage

- **Precautionary Statements**
  - **P210**: Keep away from heat, sparks, open flames and/or hot surfaces. No smoking.
  - **P233**: Keep container tightly closed.
  - **P260**: Do not breathe mists, vapors, and/or spray.
  - **P264**: Wash thoroughly after handling.
  - **P280**: Wear protective gloves/protective clothing/eye protection/face protection.
  - **P370+P378**: In case of fire: Use appropriate media for extinction.
  - **P304+P340**: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - **P310**: Immediately call a POISON CENTER or doctor/physician.
  - **P303+P361+P353**:IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower.
  - **P363**: Wash contaminated clothing before reuse.
  - **P305+P351+P338**: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - **P301+P330+P331**: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
  - **P501**: Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

G. Specimen Collection and Transport

Follow your institution's protocol for sample collection.

Sample Collection, Transport and Storage

Collection

Collect sputum or aerosol-induced sputum following your institution's standard procedures. Test unprocessed sputum or concentrated/decontaminated sputum sediment. See Table 1 to determine adequate specimen volume.

Table 1. Required Specimen Volume

Specimen Type	Minimum Volume for One Test	Maximum sample volume	Sample to Sample Reagent (SR) Ratio
Sputum sediment	0.5 mL	2.5 mL	1:3[a]
Unprocessed sputum	1 mL	4.0 mL	1:2

a. 1:2 sample to SR ratio should be used with sample volume of 0.7 mL or greater for one test.

Storage and Transport

Sputum sediment: Store resuspended sediment at 2 – 8 °C for up to seven days.

Unprocessed sputum: Transport and store sputum at 2 – 8 °C before processing whenever possible. If necessary, unprocessed sputum specimens can be stored at a maximum of 35 °C for up to three days and then at 2 – 8 °C for an additional seven days.

H. Assay Procedure

H.1 Procedure for Decontaminated, Concentrated Sputum Sediments

Note Reject specimens with obvious food particles or other solid particulates.

Volume Requirements: Sputum sediments prepared according to the method of Kent and Kubica[11] and re-suspended in 67 mM Phosphate/H_2O buffer) can be tested using the Xpert MTB/RIF Ultra Assay. After resuspension, keep at least 0.5 mL of the resuspended sediment for the Xpert MTB/RIF Ultra Assay. For all volumes less than 0.7 mL perform steps 1 – 6. These steps require 3 parts Sample Reagent (SR) to 1 part sediment in order to generate adequate volume (~2 mL) for the optimum performance of the assay.

If the sample volume is equal to or greater than 0.7 mL, adequate test volume can be produced by adding 2 parts SR to 1 part sediment. In this example 1.4 mL of SR would be added to 0.7 mL sediment. These volumes scale at a ratio of 2 parts SR to 1 part sediment.

1. Bring the cartridge to room temperature. Label each Xpert MTB/RIF Ultra cartridge with the Sample ID. See Figure 1.

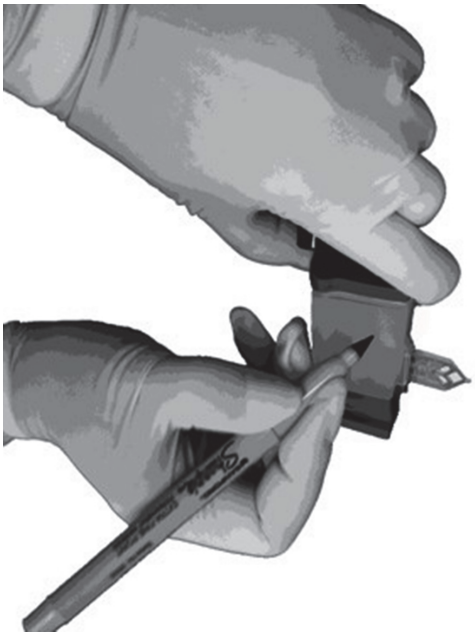


Figure 1. Write on Side of Cartridge

Note Write on the side of the cartridge or affix an ID label. Do not put the label on the lid of the cartridge or over the existing 2D barcode on the cartridge.

2. Mix the sediment by vortexing or use a pipette to aspirate and eject the material enough times to assure that all organisms are in suspension.
3. Transfer 0.5 mL of the total resuspended pellet to a conical, screw-capped tube for the Xpert MTB/RIF Ultra using a transfer pipette.

Note Store re-suspended sediments at 2 to 8 °C if they are not immediately processed. Do not run the Xpert MTB/RIF Ultra test on a re-suspended sediment that has been refrigerated for >7 days.

4. Transfer 1.5 mL of Xpert MTB/RIF Ultra Sample Reagent (SR) to 0.5 mL of resuspended sediment using a transfer pipette. Tighten cap securely.
5. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

Note One back-and-forth-movement is a single shake.

6. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds.
7. Incubate the sample at room temperature for an additional 5 minutes.

H.2 Procedure for Unprocessed Sputum

Volume Requirement: ≥1 mL of unprocessed sputum is required.

1. Bring the cartridge to room temperature. Label each Xpert MTB/RIF Ultra cartridge with the Sample ID. See Figure 1.

Note Write on the side of the cartridge or affix an ID label. Do not put the label on the lid of the cartridge or over the existing 2D barcode on the cartridge.

2. After receiving the sample in a leak-proof sputum collection container, carefully open the lid of the sputum collection container and examine the contents to be sure there are no food particles or other solid particles. See Figure 2.

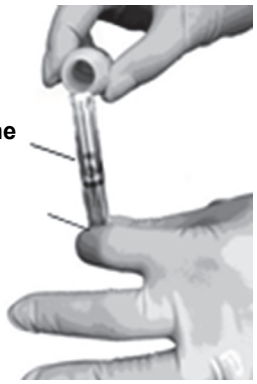
Note Reject specimens with obvious food particles or other solid particulates.

Figure 2. Opened Sample Container

3. Pour approximately 2 times the volume of the SR into the sputum (2:1 dilution, SR:sputum). See Figure 3.

Example 1
8 mL Sample Reagent:
4 mL sputum

3 mL line



Example 2
2 mL Sample Reagent:1 mL sputum

Note: Discard the leftover Sample Reagent and the bottle in a chemical waste container.

Figure 3. Examples of 2:1 Dilutions

4. Replace and secure the lid. Shake vigorously 10 to 20 times or vortex for at least 10 seconds.

Note One back-and-forth-movement is a single shake.

5. Incubate the sample for 10 minutes at room temperature.
6. Shake the specimen vigorously 10 to 20 times or vortex for at least 10 seconds. Incubate the sample at room temperature for an additional 5 minutes.

Note Ensure that the specimen is liquefied completely. If specimen is not liquefied, repeat step 6.

H.3 Preparing the Cartridge

Note If using a GeneXpert Dx instrument, start the test as soon as possible and within four hours of adding the Sample Reagent-treated sample to the cartridge. Once the sample is added to the cartridge, the cartridge should remain at room temperature prior to starting the test within four hours. If using a GeneXpert Infinity system, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the Sample Reagent-treated sample to the cartridge. Remaining shelf-life is tracked by the Xpertise software so that tests are run prior to the four hour on-board expiration.

1. Open the cartridge lid, and then open the sample container.
2. Using the provided transfer pipette, aspirate the liquefied sample to just above the line on the pipette. See Figure 4. Do not process the sample further if there is insufficient volume.

Figure 4. Aspirating to the Line on the Pipette

3. Transfer the sample into the sample chamber of the Xpert MTB/RIF Ultra cartridge. Dispense the sample slowly to minimize the risk of aerosol formation. See Figure 5.

Figure 5. Dispensing Decontaminated Liquefied Sample into the Sample Chamber of the Cartridge

4. Close the cartridge lid firmly. Remaining liquefied sample may be kept for up to 4 hours at 2 to 8 °C in case retesting is required.

H.4 Starting the Test

Important **If you are running a GeneXpert Dx system, before you start the test, make sure that the system is running GeneXpert Dx software version 4.7b or higher and that the Xpert MTB/RIF Ultra assay definition file is imported into the software.**

Important **If you are running a GeneXpert Infinity system, before you start the test, make sure that the system is running Xpertise software version 6.4b or higher and that the Xpert MTB/RIF Ultra assay definition file is imported into the software.**

This section lists the basic steps for running the test. 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:
   - If using the GeneXpert Dx instrument, first turn on the GeneXpert Dx instrument, and then turn on the computer. The GeneXpert software will launch automatically.

     or

   - If using the GeneXpert Infinity instrument, power up the instrument. On the Windows® desktop, double-click the Xpertise software shortcut icon on the Windows desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** and **Order Test** (Infinity).
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the **View Results** window and all the reports.
5. 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 the reports.

6. Scan the barcode on the Xpert MTB/RIF Ultra cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). In the dialog box that appears, enter your password, if required.
8. 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. Then remove the cartridge.
   D. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

H.5 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 model being used.

Note If reporting results using an LIS, confirm that LIS results match system results for the patient ID field; if results conflict, report the system results only.

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.

I. Quality Control

CONTROL

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

SPC—Ensures that the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB. The SPC verifies that lysis of MTB has occurred if the organisms are present and verifies that specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the real-time PCR assay.

The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be “Invalid” if the SPC is not detected in a negative test.

PCC—Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

J. Interpretation of Results

The GeneXpert Instrument System generates the results from measured fluorescent signals and embedded calculation algorithms. The results can be seen in the **View Results** window. See Figure 6, Figure 7 and Figure 8 for specific examples, and see Table 2 for a list of all possible results.

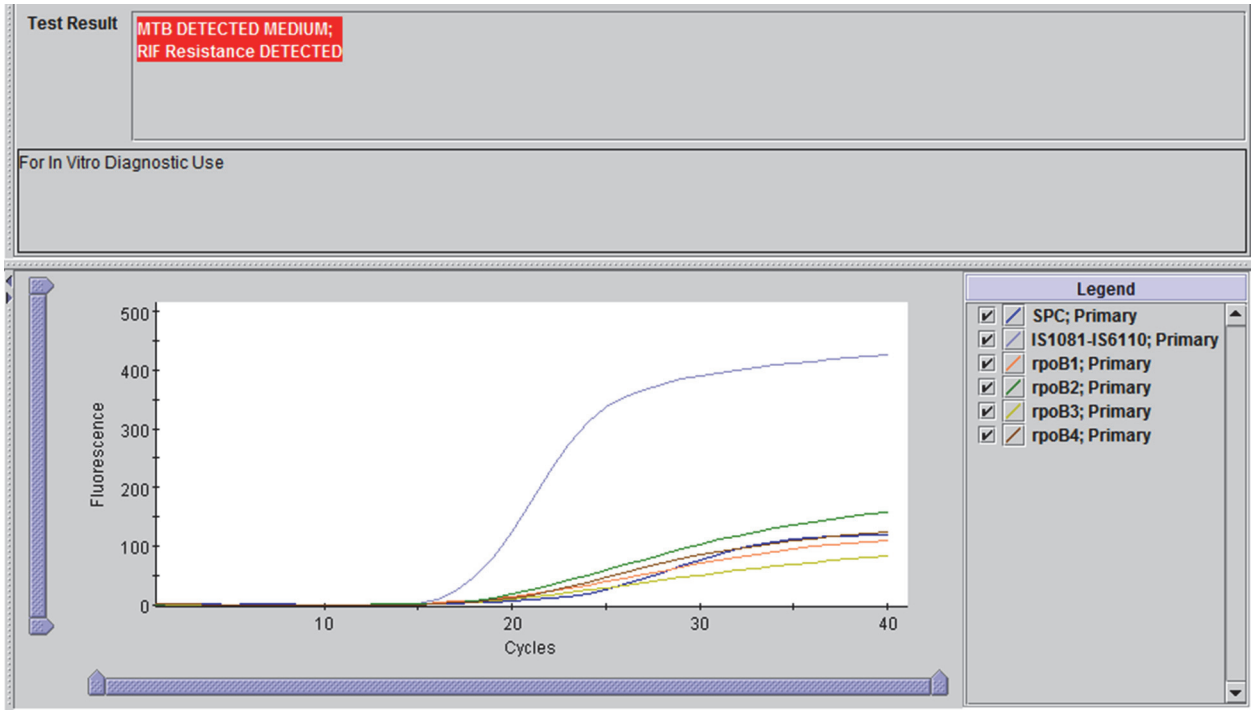


Figure 6. MTB DETECTED MEDIUM; RIF Resistance DETECTED (GeneXpert Dx Detailed User View)

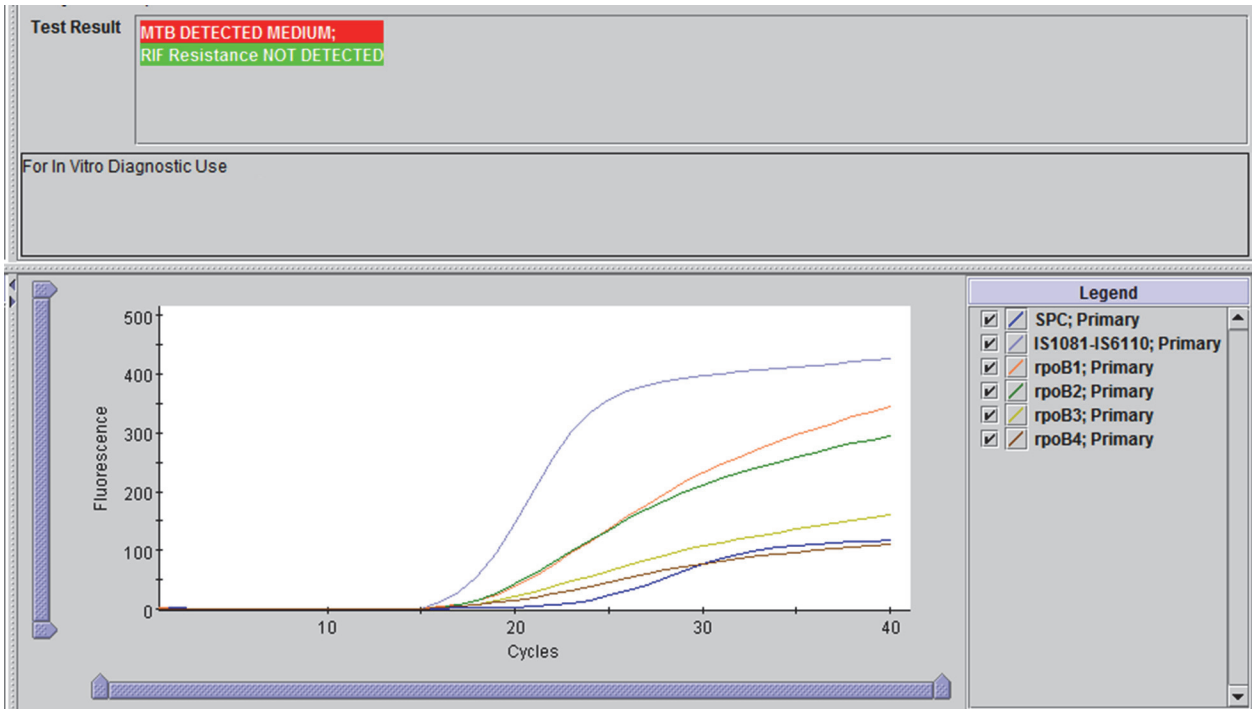


Figure 7. MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED (GeneXpert Dx Detailed User View)

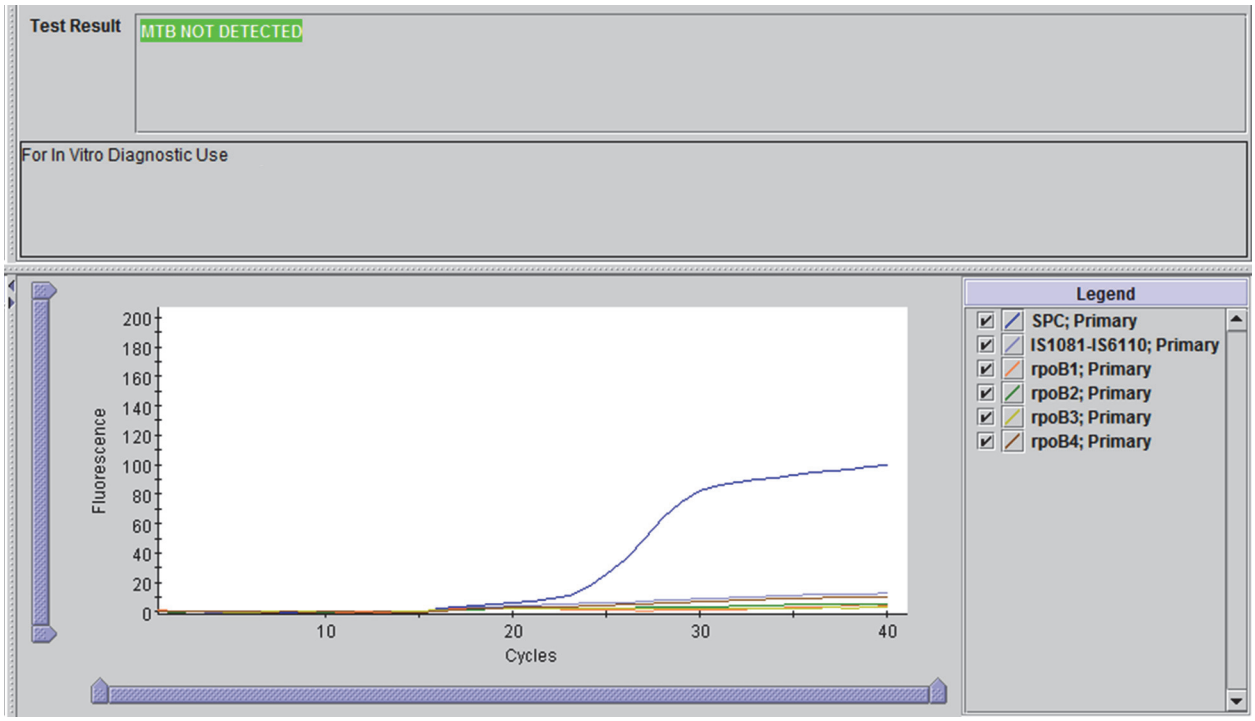


Figure 8. MTB NOT DETECTED (GeneXpert Dx Detailed User View)

Table 2. Xpert MTB/RIF Ultra Assay Results and Interpretation

Result	Interpretation
MTB DETECTED HIGH; RIF Resistance DETECTED	The MTB target is present within the sample: • A mutation in the *rpoB* gene target sequence has been detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB DETECTED MEDIUM; RIF Resistance DETECTED	
MTB DETECTED LOW; RIF Resistance DETECTED	
MTB DETECTED VERY LOW; RIF Resistance DETECTED	
MTB DETECTED HIGH; RIF Resistance NOT DETECTED	The MTB target is present within the sample: • No mutation in the *rpoB* gene target sequence has been detected. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB DETECTED MEDIUM; RIF Resistance NOT DETECTED	
MTB DETECTED LOW; RIF Resistance NOT DETECTED	
MTB DETECTED VERY LOW; RIF Resistance NOT DETECTED	

Table 2. Xpert MTB/RIF Ultra Assay Results and Interpretation (Continued)

Result	Interpretation
MTB DETECTED HIGH; RIF Resistance INDETERMINATE **MTB DETECTED MEDIUM; RIF Resistance INDETERMINATE** **MTB DETECTED LOW; RIF Resistance INDETERMINATE** **MTB DETECTED VERY LOW; RIF Resistance INDETERMINATE**	The MTB target is present within the sample: • RIF resistance could not be determined due to invalid melt peaks. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB Trace DETECTED; RIF Resistance INDETERMINATE	The MTB target is present within the sample: • RIF resistance cannot be determined due to insufficient signal detection. • SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control. • Probe Check: PASS. All probe check results pass.
MTB NOT DETECTED	The MTB target is not detected within the sample: • SPC: PASS. The SPC met the acceptance criteria. • Probe Check: PASS. All probe check results pass.
INVALID	The presence or absence of MTB cannot be determined. The SPC does not meet the acceptance criteria, the sample was not properly processed, or PCR was inhibited. Repeat the test. See the Retest Procedure section of this document. • MTB INVALID: The presence or absence of MTB DNA cannot be determined. • SPC: FAIL. The MTB target result is negative, and the SPC Ct is not within valid range. • Probe Check: PASS. All probe check results pass.
ERROR	The presence or absence of MTB cannot be determined. Repeat the test. See the Retest Procedure section of this document. • MTB: NO RESULT • SPC: NO RESULT • Probe Check: FAIL. All or one of the probe check results failed. **Note:** If the probe check passed, the error is caused by a system component failure.
NO RESULT	The presence or absence of MTB cannot be determined. Repeat the test. See the Retest Procedure section of this document. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • MTB: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

Table 3. Xpert MTB/RIF Ultra Assay: All Possible Results

TB Results	RIF Results
MTB DETECTED HIGH	RIF Resistance DETECTED
MTB DETECTED HIGH	RIF Resistance NOT DETECTED
MTB DETECTED HIGH	RIF Resistance INDETERMINATE
MTB DETECTED MEDIUM	RIF Resistance DETECTED
MTB DETECTED MEDIUM	RIF Resistance NOT DETECTED
MTB DETECTED MEDIUM	RIF Resistance INDETERMINATE
MTB DETECTED LOW	RIF Resistance DETECTED
MTB DETECTED LOW	RIF Resistance NOT DETECTED
MTB DETECTED LOW	RIF Resistance INDETERMINATE
MTB DETECTED VERY LOW	RIF Resistance DETECTED
MTB DETECTED VERY LOW	RIF Resistance NOT DETECTED
MTB DETECTED VERY LOW	RIF Resistance INDETERMINATE
MTB TRACE[a] DETECTED	RIF Resistance INDETERMINATE
MTB NOT DETECTED	
INVALID	
ERROR	
NO RESULT	

a. A TRACE result call means that low levels of MTB are detected but no RIF resistant result is detected. This occurs due to the increased sensitivity of TB detection using multi-copy targets IS6110 and IS1081 as opposed to RIF resistance detection using the single copy *rpoB* gene. Therefore a RIF resistant or susceptible result cannot be determined in a TRACE sample. The TRACE sample is always **RIF Resistance INDETERMINATE**.

J.1 Reasons to Repeat the Assay

Repeat the test using a new cartridge if one of the following test results occurs.

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

J.2 Retest Procedure

If you have leftover fresh sputum or reconstituted sediment, always use new SR to decontaminate and liquefy the sputum or the sediment before running the assay. See Section H., Assay Procedure or Section H.2, Procedure for Unprocessed Sputum.

If you have a sufficient leftover SR-treated sample and are within 4 hours of the initial addition of SR to the sample, you can use the leftover sample to prepare and process a new cartridge. When retesting, always use a new cartridge and start the test immediately. See Section H.3, Preparing the Cartridge.

K. Limitations

Because the detection of MTB is dependent on the number of organisms present in the sample, reliable results are dependent on proper sample collection, handling, and storage. Erroneous test results might occur from improper sample collection, handling or storage, technical error, sample mix-up, or an insufficient concentration of starting material. Careful compliance to the instructions in this insert is necessary to avoid erroneous results.

A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of MTB and Rifampin resistance.

Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MDR-MTB or rifampin resistant strains resulting in a false rifampin-sensitive result.

The Xpert MTB/RIF Ultra Assay performance has not been evaluated in patients less than eighteen years of age.

The Xpert MTB/RIF Ultra Assay does not provide confirmation of rifampin susceptibility since mechanisms of rifampin resistance other than those detected by this device may exist that may be associated with a lack of clinical response to treatment. Specimens that have both MTB-complex DNA and rifampin-resistance associated mutations of the *rpoB* gene detected by the Xpert MTB/RIF Ultra Assay should be considered for additional drug susceptibility testing.

The performance of the Xpert MTB/RIF Ultra test is dependent on operator proficiency and adherence to assay procedures. Assay procedural errors may cause false positive or false negative results. All device operators should have appropriate device training.

L. Clinical Performance Characteristics

This section lists the performance characteristics of the Xpert MTB/RIF Ultra Assay.

L.1 Clinical Study Design

The performance characteristics of the Xpert MTB/RIF Ultra Assay were evaluated for the detection of MTB-complex DNA and for the detection of RIF-resistance associated mutations in sputum specimens relative to results from culture (solid and/or liquid media) and drug susceptibility testing (DST), respectively. This multi-center study used prospective and archived direct (raw) sputum or concentrated sediment specimens collected from subjects 18 years or older. Subjects included pulmonary TB suspects on no TB treatment or less than 3 days of treatment within 6 months of the study start (TB suspects) as well as previously TB treated subjects who were suspected of multi-drug resistant TB (MDR TB suspects). The study was conducted worldwide (Belarus, Brazil, China, Georgia, Germany, India, Italy, Kenya, Peru, South Africa, Uganda, Vietnam and the United States). The sensitivity and specificity of the Xpert MTB/RIF Ultra assay for MTB detection were evaluated using data from only the TB suspects; whereas the data from the MDR TB suspects were combined to evaluate the performance of RIF resistance.

The specimens came from study subjects, 61% male (n=1111), 35% female (n=648); for 4% (n=76) gender was unknown. They were from geographically diverse regions: 12% (n=217) were from the US (California, New York and Florida) and 88% (n=1618) were from countries outside the US (Belarus, Brazil, China, Georgia, Germany, India, Italy, South Africa, Kenya, Peru, Vietnam and Uganda). Of the 1835 specimens, 1228 were prospectively collected and 607 were from frozen archived specimen banks.

L.2 Xpert MTB/RIF Ultra Assay Performance vs. MTB Culture

Up to three sputum specimens were collected from each study subject for use in the clinical study. For prospective specimens, the first sputum specimen was tested by the Xpert MTB/RIF Ultra Assay and the second two specimens were used for TB culture. For archived specimens, culture results were available from the standard of care method and Xpert MTB/RIF Ultra Assay was performed using the first specimen with sufficient volume. If the assay result was non-determinate (**ERROR**, **INVALID** or **NO RESULT**), the specimen was retested if there was sufficient volume. Overall, 1.0% of tested samples from eligible subjects (19/1854; 95% CI: 0.7, 1.6) were non-determinate. The acid fast bacilli (AFB) smear status for a subject was determined by Auramine-O (AO) fluorescent or Ziehl-Neelsen (ZN) smear stain from the specimen with the corresponding Xpert MTB/RIF Ultra Assay result. The MTB culture status for all subjects was defined based on the MTB culture result of all specimens collected within a seven day period for that subject.

The performance of the Xpert MTB/RIF Ultra Assay for detection of MTB relative to MTB culture, stratified by AFB smear status, is shown in Table 4. The sensitivity in smear positive and smear negative specimens was 99.5% (426/428), 95% CI: 98.3, 99.9 and 73.3% (200/273), 95% CI: 67.7, 78.2, respectively. The overall specificity of the Xpert MTB/RIF Ultra Assay regardless of AFB smear was 95.5% (1222/1280), 95% CI: 94.2, 96.5.

Table 4. Xpert MTB/RIF Ultra Assay Performance vs. MTB Culture

		Smear/Culture				
		Positive			Negative	Total
		AFB Smear +	AFB Smear -	Overall Culture +	Overall Culture -	
Xpert MTB/RIF Ultra Assay	MTB DETECTED	426	200	630[a]	58	688
	MTB NOT DETECTED	2	73	75	1222	1297
	Total	428	273	705	1280	985
Performance in Smear Positive:						
Sensitivity: 99.5% (426/428), 95% CI: 98.3, 99.9						
Performance in Smear Negative:						
Sensitivity: 73.3% (200/273), 95% CI: 67.7, 78.2						
Performance Overall:						
Sensitivity: 89.5% (630/705), 95% CI: 86.9, 91.4						
Specificity: 95.5% (1222/1280), 95% CI: 94.2, 96.5						

a. Smear results were not available for 4 culture positive specimens.

The performance of the Xpert MTB/RIF Ultra Assay for detection of MTB relative to MTB culture, stratified by Non-US vs. US sites is shown in Table 5. Among 1985 specimens, there were 1768 specimens from Non-US sites and 217 from US sites.

Table 5. Xpert MTB/RIF Ultra Assay vs. MTB Culture by Non-US vs. US Sites

	Non-US		US	
	N	Percent (95% CI)	N	Percent (95% CI)
Sensitivity Smear Pos	380/382	99.5% (98.1,99.9)	46/46	100.0% (92.3, 100)
Sensitivity Smear Neg	180/245	73.5% (67.6, 78.6)	20/28	71.4% (52.9, 84.7)
Overall Sensitivity	564/631[a]	89.4% (86.7, 91.6)	66/74	89.2% (80.1, 94.4)
Overall Specificity	1080/1137	95.0% (93.6, 96.1)	142/143	99.3% (96.1, 99.9)

a. Smear results were not available for 4 culture positive specimens.

L.3 Xpert MTB/RIF Ultra Assay Performance vs. Culture by Smear Type

The performance of the Xpert MTB/RIF Ultra Assay for detection of MTB was determined relative to MTB culture in specimens with AFB smear performed by AO and ZN. Results are shown in Table 6. Among 1985 specimens, there were 1810 specimens with AO smears and 175 with ZN smears.

Table 6. Performance of Xpert MTB/RIF Ultra Assay vs. MTB Culture by Auramine O (AO) and Ziehl-Neelsen (ZN) Staining Methods

	Auramine O Method		Ziehl-Neelsen Method	
	N	Percent (95% CI)	N	Percent (95% CI)
Sensitivity Smear Pos	386/388	99.5% (98.1,99.9)	40/40	100% (91.2, 100)
Sensitivity Smear Neg	153/219	69.9% (63.5, 75.6)	47/54	87.0% (75.6, 93.6)
Overall Sensitivity	543/611[a]	88.9% (86.1, 91.1)	87/94	92.6% (85.4, 96.3)
Overall Specificity	1145/1199	95.5% (94.2, 96.5)	77/81	95.1% (88.0, 98.1)

a. Smear results were not available for 4 culture positive specimens.

L.4 Xpert MTB/RIF Ultra Assay Performance vs. Culture by Specimen Type

The performance of the Xpert MTB/RIF Ultra Assay for detection of MTB was determined relative to MTB culture in unprocessed sputum and concentrated sputum sediment specimens. Results are shown in Table 7. Among 1895 specimens, there were 1543 unprocessed sputum specimens and 442 concentrated sputum sediment specimens.

Table 7. Xpert MTB/RIF Ultra Assay vs. MTB Culture by Specimen Type

	Direct Sputum		Sputum Sediments	
	N	% (95% CI)	N	% (95% CI)
Sensitivity Smear Pos	323/324	99.7% (98.2,99.9)	103/104	99.0% (94.8, 99.8)
Sensitivity Smear Neg	168/229	73.5% (67.2, 78.9)	32/44	72.7% (58.2, 83.7)
Overall Sensitivity	495/557[a]	88.9% (86.0, 91.2)	135/148	91.2% (85.6, 94.8)
Overall Specificity	937/986	95.0% (93.5, 96.2)	285/294	96.9% (94.3, 98.4)

a. Smear results were not available for 4 culture positive specimens.

L.5 Xpert MTB/RIF Ultra Assay Performance vs. Drug Susceptibility Testing for RIF

MTB positive culture isolates were tested for drug susceptibility (DST) to rifampin using the agar proportion method with Middlebrook or Lowenstein-Jensen media, the Thermo Scientific Sensititre™ Mycobacterium tuberculosis MIC Plate or the BD BACTEC™ MGIT™ 960 SIRE assay. The performance of the Xpert MTB/RIF Ultra Assay for detection of RIF-resistance associated mutations was determined relative to the DST results of the MTB culture isolates.

Results for the detection of RIF resistance associated mutations are reported by the Xpert MTB/RIF Ultra Assay only when the *rpo*B gene sequence of MTB-complex was detected by the device. The performance of RIF susceptibility/resistance are reported in Table 8. Specimens with DST not done, **MTB NOT DETECTED** and **MTB DETECTED; RIF RESISTANCE INDETERMINATE** were excluded from the analysis**.** Sixty-three (63) of 67 specimens with RIF indeterminate results were **MTB DETECTED TRACE; RIF RESISTANCE INDETERMINATE**.

Table 8. Xpert MTB/RIF Ultra Performance vs. DST

		Drug Susceptibility Test		
		RIF Resistant	**RIF Susceptible**	**Total**
Xpert MTB/RIF Ultra	**MTB DETECTED; RIF Resistance DETECTED**	128	12[a]	140
	MTB DETECTED; RIF Resistance NOT DETECTED	5[b]	314	319
	Total	133	326	459
		Sensitivity: 96.2% (128/133), 95% CI: 91.5, 98.4 Specificity: 96.3% (314/326), 95% CI: 93.7, 97.9		

a. Discrepant sequencing results: 11 of 12 RIF resistant, 1 of 12 not available.
b. Discrepant sequencing results: 4 of 5 RIF susceptible, 1 of 5 not available.

L.6 Xpert MTB/RIF Ultra Assay Performance vs. the Xpert MTB/RIF Assay

One thousand five hundred ninety-four (1594) specimens were tested by both the Xpert MTB/RIF Ultra Assay and the Xpert MTB/RIF Assay. The overall percent agreement between the assays was 96.5% [(1538/1594) 95% CI: 95.5, 97.3]. The positive percent agreement and the negative percent agreement were 99.2% [(491/495) 95% CI: 97.9, 99.7] and 95.3% [(1047/1099) 95% CI: 93.8, 96.4], respectively.

M. Analytical Performance Characteristics

M.1 Interfering Substances

A study was performed in artificial sputum matrix to assess the effects of potential interfering substances with the Xpert MTB/RIF Ultra Assay. A total of 32 potentially interfering substances were evaluated. Potentially endogenous interfering substances may include, but are not limited, to blood, pus (white blood cells), cells from the respiratory tract, mucin, human DNA, and gastric acid from the stomach. Other potentially interfering substances may include anesthetics, antibiotics, antibacterial, anti-tuberculosis drugs, anti-viral drugs, bronchodilators, inhaled bronchodilators, live intranasal influenza virus vaccine, germicidal mouthwash, specimen processing reagents, *Pneumocystis jiroveci* medication, homeopathic allergy relief medications, nasal corticosteroids, nasal gels, nasal sprays, oral anesthetics, oral expectorants, neutralizing buffers, and tobacco. These substances are listed in Table 9 with active ingredients and concentrations tested shown. Positive and negative samples were included in this study. Positive samples were tested near at 3 times the analytical limit of detection using BCG cells in replicates of 8. Negative samples, comprised of the substance absent the MTB strain, were tested per substance in replicates of 8 to determine the effect on the performance of the sample processing control (SPC).

No inhibitory effect was observed for any of the 32 potentially interfering substances tested (Table 9).

Table 9. Interfering Substances

Substance	Description/Active Ingredient	Concentration Tested
Blood	Blood (human)	5% (v/v)
Germicidal Mouthwash	Chlorhexidine gluconate (0.12%), 20% solution	20% (v/v)
Specimen Processing Reagents	Cetylpyridinium chloride, 1% in 2% NaCl	0.5% (v/v) in 1% NaCl
Specimen Processing Reagents	Cetylpyridinium chloride, 1% in 2% NALC	0.5% (v/v) in 1% NALC
Specimen Processing Reagents	Cetylpyridinium chloride, 1% in 2% NALC plus 25 mM Citrate	0.5% (v/v) in 1% NALC plus 12.5 mM Citrate
Gastric Acid	pH 3 to 4 solution in water, neutralized with sodium bicarbonate	100% (v/v)
Human DNA/Cells	HELA 229	10^6 cells/mL
Antimycotic; Antibiotic	Nystatin oral suspension, 20%	20% (v/v)
White Blood Cells (human)	WBC/Pus matrix (30% buffy coat; 30% plasma; 40% PBS)	100% (v/v)
Anesthetics (endotracheal intubation)	Lidocaine HCl 4%	30% (v/v)
Nebulizing solutions	NaCl 5% (w/v)	5% (w/v)
Mucin	Mucin 5% (w/v)	5% (w/v)
Antibacterial, systemic	Levofloxacin 25 mg/mL	5 mg/mL
Nasal corticosteroids	Fluticasone 500 mcg/spray	5 µg/mL
Inhaled bronchodilators	Albuterol Sulfate 2.5 mg/3mL	100 µg/mL
Oral anesthetics	Orajel (20% Benzocaine)	5% (w/v)
Anti-viral drugs	Acyclovir, IV 50 mg/mL	50 µg/mL
Antibiotic, nasal ointment	Neosporin (400U Bacitracin, 3.5 mg Neomycin, 5000U Polymyxin B)	5% (w/v)
Tobacco	Nicogel (40% tobacco extract)	0.5% (w/v)
Anti-tuberculosis drugs	Streptomycin 1mg/mL	25 µg/mL
Anti-tuberculosis drugs	Ethambutol 1 mg/mL	50 µg/mL
Anti-tuberculosis drugs	Isoniazid 1 mg/mL	50 µg/mL
Oral expectorants	Guaifenesin (400mg/tablet)	5 mg/mL
Anti-tuberculosis drugs	Pyrazinamide 10 mg/mL	10 µg/mL
Nasal gel (Homeopathic)	Zicam gel	50% (w/v)
Nasal spray	Phenylephrine 0.5%	1% (v/v)
Anti-tuberculosis drugs	Rifampicin 1mg/mL	25 µg/mL
Allergy relief medicine (Homeopathic)	Tea tree oil (<5% Cineole, >35% Terpinen- 4-01)	0.5% (v/v)
Live intranasal influenza virus vaccine	Live influenza virus vaccine FluMist	5% (v/v)
Pneumocystis jiroveci medication	Pentamidine	300 ng/mL (v/v)
Bronchodilator	Epinephrine (injectable formulation)	1 mg/mL
Anti-tuberculosis drugs	Amoxicillin	25 µL

M.2 Analytical Sensitivity

Additional studies were performed to determine the 95% confidence interval for the analytical limit of detection (LoD) of this assay. The limit of detection is defined as the lowest number of colony forming units (CFU) per sample that can be reproducibly distinguished from negative samples with 95% confidence. The analytical LoD was determined by testing 20 replicates of different concentrations of *M. tuberculosis* (H37Rv) cells spiked into negative clinical sputum samples.

Under the conditions of the study, results indicate that the LoD point estimate for *M. tuberculosis* is 11.8 CFU/mL with a 95% confidence interval ranging from 8.6 CFU to 15 CFU. The estimate and confidence levels were determined using probit analysis with data (number of positives per number of tests at each level) taken at different concentrations.

M.3 Analytical Specificity (Exclusivity)

Cultures of 30 nontuberculous mycobacteria (NTM) strains were tested with the Xpert MTB/RIF Ultra Assay. Three replicates of each isolate were spiked into buffer and tested at a concentration of $\geq 10^7$ CFU/mL. See Table 10.

Table 10. NTM Strains Tested for Specificity

Mycobacterium avium* subsp. *avium	***Mycobacterium scrofulaceum***
Mycobacterium celatum	***Mycobacterium simiae***
Mycobacterium chelonae	***Mycobacterium szulgai***
Mycobacterium gordonae	***Mycobacterium thermoresistibile***
Mycobacterium haemophilum	***Mycobacterium triviale***
Mycobacterium abscessus	***Mycobacterium vaccae***
Mycobacterium asiaticum	***Mycobacterium xenopi***
Mycobacterium flavescens	***Mycobacterium smegmatis***
Mycobacterium fortuitum* subsp. *fortuitum	***Mycobacterium interjectum***
Mycobacterium gastri	***Mycobacterium peregrinum***
Mycobacterium genavense	***Mycobacterium mucogenicum***
Mycobacterium intracellulare	***Mycobacterium goodii***
Mycobacterium kansasii	***Mycobacterium shimodei***
Mycobacterium malmoense	***Mycobacterium phlei***
Mycobacterium marinum	***Mycobacterium terrae***

Under the conditions of the study, all of the NTM isolates were reported as **MTB NOT DETECTED**. Positive and negative controls were included in the study. The specificity was 100%.

Additionally, in order to determine if high concentrations of NTM would interfere with the detection of low levels of TB, six of the strains listed in Table 10 were mixed with the TB strain H37Rv in sputum to a final concentration of 10^6 CFU/mL NTM and 36 CFU/mL H37Rv.

NTM strains tested for ability to interfere with TB (H37Rv) detected included:

- *M. abscessus*, ATCC 19977
- *M. avium* National Jewish Hospital clinical isolates
- *M. celatum*, National Jewish Hospital clinical isolates
- *M. kansasii*, ATCC 12478
- *M. gordonae*, ATCC 14470
- *M. intracellulare*, National Jewish Hospital clinical isolates

The tested NTM strains did not interfere with the detection of 36 CFU/mL of *M. tuberculosis*; thus, the signals were the same as when H37Rv was tested alone.

M.4 Species/Strains Tested for Specificity

The following microorganisms including Gram-negative bacteria, Gram-positive bacteria, fungal organisms, viruses and yeast were tested for false positivity in the Xpert MTB/RIF Ultra Assay. The replicates of each isolate were spiked onto buffer and tested at a concentration of $\geq 10^7$ CFU/mL (bacteria and fungal strains) or $\geq 10^6$ copies/mL (genomic DNA for bacteria and fungi) and $\geq 10^5$ $TCID_{50}$ /mL (virus strains).

Table 11. Species and Strains

Acinetobacter baumannii	***Klebsiella pneumoniae***	***Respiratory Syncytial Virus Type B***
Aspergillus fumigatus	***Moraxella catarrhalis***	***Rhinovirus***
Candida albicans	***Neisseria meningitidis***	***Staphylococcus aureus***
Chlamydophila pneumoniae	***Neisseria mucosa***	***Staphylococcus epidermidis***
Citrobacter freundii	***Nocardia asteroides***	***Stenotrophomonas maltophilia***
Corynebacterium xerosis	***Parainfluenza Virus Type 1***	***Streptococcus agalactiae***
Coronavirus	***Parainfluenza Virus Type 2***	***Streptococcus mitis***
Enterobacter cloacae	***Parainfluenza Virus Type 3***	***Streptococcus mutans***
Escherichia coli	***Pseudomonas aeruginosa***	***Streptococcus pneumoniae***
Haemophilus influenzae	***Respiratory Syncytial Virus Type A***	***Streptococcus pyogenes***
Human metapneumovirus (hMPV) 16 Type A1		

Under the conditions of the study, all of the microorganisms tested were reported as **MTB NOT DETECTED**. Positive and negative controls were included in the study. The specificity was 100%.

M.5 Analytical Inclusivity

Thirty-seven MTB-complex strains consisting of 16 rifampin-susceptible strains with a wild-type *rpoB* core region and 21 rifampin-resistant strains were tested using the Xpert MTB/RIF Ultra Assay. DNA samples from a total of 37 MTB strains were tested on the GeneXpert using an Xpert MTB/RIF Ultra protocol modified for DNA testing. The final reaction components and PCR cycling conditions were unchanged from the protocol designed for patient sample testing. Twelve of the strains were from the WHO/TDR collection and 6 from the laboratory collection at Rutgers University. Collectively these strains represent isolates from 8 countries and contained 21 RIF-resistant isolates comprised of single, double and one triple *rpoB* core region mutations. The samples were tested by adding 100 µL of the DNA sample to the lysate chamber of the cartridge. The negative reactions used buffer as the sample. The assay correctly identified all 16 wild-type strains and correctly identified rifampin resistance in 18 of 21 strains resistant to rifampin with mutations in the *rpoB* core region. Indeterminate rifampin results were obtained for 3 mutant strains.

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O. Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408.541.4191	Telephone: +33 563 825 300
Fax: +1 408.541.4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

P. Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

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

Region	Telephone	Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 107 884 + 0800 001 028	techsupportANZ@cepheid.com
China	+ 86 021 5406 5387	techsupportchina@cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Germany	+ 49 69 710 480 480	support@cepheideurope.com
United Kingdom	+ 44 3303 332 533	support@cepheideurope.com
Italy	+ 39 800 902 567	support@cepheideurope.com
South Africa	+ 27 861 22 76 35	support@cepheideurope.com
Other European, Middle East and African countries	+ 33 563 825 319 + 971 4 253 3218	support@cepheideurope.com
Japan	+ 0120 95 4886	support@japan.cepheid.com
India	+91 11 48353 010	techsupportindia@cepheid.com
Other countries not listed	+ 1 408 400 8495	techsupport@cepheid.com

Contact information for other Cepheid offices is available on our website at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab. Select the **Contact Us** option.

Q. Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not re-use
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
CE	CE marking – European Conformity
	Temperature limitation
	Biological risks
	Flammable Liquids
	Skin Corrosion
	Warning

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

Dalinis dokumento vertimas iš anglų kalbos

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® MTB/RIF Ultra

REF **GXMTB/RIF-ULTRA-10**
GXMTB/RIF-ULTRA-50

Cepheid

In Vitro diagnostinė medicinos priemonė

CE IVD

301-5987, Redakcija D, 2017 m. gegužės mėn.

Xpert® MTB/RIF Ultra

Tik *In Vitro* diagnostiniam naudojimui.

Patentuotas pavadinimas
Xpert® MTB/RIF Ultra

Bendrinis / įprastinis pavadinimas
Xpert® MTB/RIF Ultra tyrimas

Paskirtis
Xpert MTB/RIF Ultra tyrimas, atliekamas su Cepheid GeneXpert instrumentų sistemomis, yra pusiau kiekybinis pakopinis (*anglų k.* nested) tikro laiko polimerazės grandinės reakcijos (PGR) *in vitro* diagnostinis tyrimas, skirtas *Mycobacterium tuberculosis* (MTB) komplekso DNR nustatymui neapdorotuose skreplių mėginiuose arba koncentruotose sedimentuose, paruoštuose iš indukuotų ar išspjautų skreplių. Mėginiai, kuriuose yra aptiktas *Mycobacterium tuberculosis* kompleksas, Xpert MTB/RIF Ultra taip pat gali aptikti atsparumą rifampinui, susisijusį su *rpoB* geno mutacijomis. Xpert MTB/RIF Ultra tyrimas yra skirtas naudoti su mėginiais, surinktais iš pacientų, kuriems yra įtariama tuberkuliozė (TB) ir kuriems nebuvo taikytas tuberkuliozės gydymas arba per pastaruosius 6 mėnesius buvo taikytas trumpesnis nei 3 dienų gydymas. Šis tyrimas yra naudojamas kaip pagalbinė priemonė atliekant plaučių tuberkuliozės diagnozę, atsižvelgiant į klinikines bei kitas laboratorines išvadas.

Procedūros principas
GeneXpert Dx sistema integruoja ir automatizuoja mėginio apdorojimą, nukleininės rūgšties amplifikaciją bei taikinio eilių aptikimą paprastuose ar kompleksiniuose mėginiuose, naudojant tikro laiko PGR ir lydymosi piko aptikimą.
Sistemą sudaro instrumentas, asmeninis kompiuteris, brūkšninių kodų skaitytuvas ir įdiegta programinė įranga, skirta mėginių tyrimų paleidimui ir rezultatų peržiūrai. Kiekvienam tyrimui yra reikalingos vienkartinio naudojimo GeneXpert kasetės, kuriose PGR reagentai ir kuriose vyksta PGR procesai. Kadangi kasetės yra individualios, kryžminio mėginių užterštumo rizika yra minimali. Pilną sistemos aprašymą rasite *GeneXpert Dx System* arba *GeneXpert Infinity System* naudotojo vadove.
Xpert MTB/RIF Ultra tyrimo sudėtyje yra reagentų, skirtų MTB ir RIF atsparumo nustatymui bei mėginio apdorojimo kontrolė (SPC), skirta adekvataus taikinio bakterijos apdorojimo atlikimui ir inhibitoriaus(-ių) stebėjimui PGR reakcijoje bei vėlesniam lydymosi piko aptikimui. Tyrimo tikrinimo kontrolė (PCC) patikrina reagento rehidraciją, PGR mėgintuvėlio užpildymą kasetėje, mėgintuvėlio integralumą ir dažų stabilumą.

Laikymas ir naudojimas

- Xpert MTB/RIF Ultra tyrimo kasetės ir reagentai turi būti laikomi prie 2–28 °C.
- Neatidarykite kasetės tol, kol nebūsite pasiruošę atlikti tyrimo.
- Nenaudokite pasibaigusios galiojimo datos reagentų ar kasečių.

Įspėjimai ir atsargumo priemonės

•Su visais biologiniais mėginiais, įskaitant panaudotas kasetes, elkitės kaip su galinčiais pernešti infekcinius agentus. Kadangi nėra žinoma, kuris mėginys yra infekciškas, su visais biologiniais mėginiais reikia dirbti laikantis universaliųjų atsargumo priemonių. Mėginių naudojimo rekomendacijas teikia JAV Ligų kontrolės ir prevencijos centrai ir Laboratorijos standartų institutas.
• Naudojant mėginius ir reagentus, dėvėkite vienkartines apsaugines pirštines, laboratorinį chalatą ir apsauginius akinius. Po kiekvieno mėginio ir reagento naudojimo, švariai nusiplaukite rankas.
• Naudojant chemikalus ir biologinius mėginius, laikykitės Jūsų laboratorijoje atliekamų saugos procedūrų.
• Nesukeiskite Xpert MTB/RIF Ultra tyrimo reagentų su kitais reagentais.
• Neatidarykite Xpert MTB/RIF Ultra kasetės dangtelio, išskyrus tą momentą, kai dedate mėginio reagentu apdorotą mėginį.
• Nenaudokite kasetės, kuri buvo išmesta ar supurtyta po išėmimo iš pakuotės.
• Nenaudokite kasetės, kuri buvo išmesta, supurtyta arba kasetės turinys yra ištekėjęs po apdoroto mėginio įdėjimo. Kasetės purtymas ar išmetimas ant žemės po atidarymo gali sukelti klaidingus rezultatus.
• Mėginio ID etiketės neklijuokite ant kasetės dangtelio ar ant brūkšninio kodo etiketės.
• Nenaudokite kasetės, jei ji yra drėgna ar pažeista jos dangtelio apsauga.
• Nenaudokite kasetės, kurios reakcijos mėgintuvėlis yra pažeistas.
• Apdorojant kelis mėginius vienu metu, kasetes atidarykite po vieną; dozuokite mėginio reagentu apdoroto mėginio ir uždarykite kasetę prieš kito mėginio apdorojimą. Tarp skirtingų mėginių ir reagentų naudojimo, švariai nusiplaukite rankas.

• Kiekviena vienkartinio naudojimo Xpert MTB/RIF Ultra tyrimo kasetė yra skirta vieno tyrimo atlikimui. Kasečių nenaudokite pakartotinai.
• Laikykitės geros laboratorijos praktikos ir po kiekvieno paciento mėginio pasikeiskite pirštines, jog išvengtumėte mėginių ar reagentų užteršimo. Darbo paviršių/vietą reguliariai valykite 10% balikliu, nušluostykite ir dar kartą nuvalykite 70% etanoliu ar izopropilo alkoholiu prieš ir po mėginių naudojimo.
• Laikykitės regioninių/šalies reikalavimų, taikomų pavojingų ir medicininių atliekų išmetimui. Jei taisyklėse nėra pateikiami aiškūs nurodymai dėl mėginių ar panaudotų kasečių išmetimo, atliekos turi būti laikomos potencialiai pernešančiomis infekcinius agentus. Panaudotas kasetes išmeskite kaip cheminio pavojaus medicinines atliekas į patvarius konteinerius, kaip nurodoma PSO (Pasaulio sveikatos organizacija) pateikiamose medicininių atliekų naudojimo ir išmetimo rekomendacijose.

Techninė pagalba

Skambinant ar rašant el. laišką į Cepheid techninės pagalbos skyrių, turėkite šią informaciją:
• Produkto pavadinimas
• Partijos numeris
• Instrumento serijos numeris
• Klaidų pranešimai (jei yra)
• Programinės įrangos versija ir, jei taikoma, kompiuterio serverio numeris.

Regionas	Telefonas	El. paštas
JAV	+1 888 838 3222	techsupport@cepheid.com
Australija ir Naujoji Zelandija	+ 1800 107 884 + 0800 001 028	techsupportANZ@cepheid.com
Kinija	+ 86 021 5406 5387	techsupportchina@cepheid.com
Prancūzija	+33 563 825 319	support@cepheideurope.com
Vokietija	+49 69 710 480 480	support@cepheideurope.com
Jungtinė Karalystė	+44 3303 332 533	support@cepheideurope.com
Italija	+ 39 800 902 567	support@cepheideurope.com
Pietų Afrika	+ 27 861 22 76 35	support@cepheideurope.com
Kitos Europos, Vidurio Rytų ir Afrikos šalys	+33 563 825 319 +971 4 253 3218	support@cepheideurope.com
Japonija	+ 0120 95 4886	support@japan.cepheideurope.com
Indija	+91 11 48353 010	techsupportindia@cepheid.com
Kitos šalys	+1 408 400 8495	techsupport@cepheid.com


Gamintojas
Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Švedija

CE IVD

Tikslus dokumento vertimas į lietuvių kalbą
Vertėja Akvilė Gegelevičienė
Data 2018-07-02

UAB Diamedica
Molėtų pl. 73, Vilnius, Lietuva
Tel. 8 5 279 0080