

Xpert® MTB/RIF

Two-hour detection of MTB and resistance to rifampicin.

*Go from test and wait
to **test and treat.***



CE IVD In vitro diagnostic medical device

defining *on-demand* molecular diagnostics.

“If you were to inoculate an Xpert[®] MTB/RIF test at the same time you started preparing your acid fast smears, by the time you finished reading the smears, the Xpert MTB/RIF test result would be ready, telling you if your positive acid fast smear was TB *and* if the strain was resistant to rifampicin, which is an excellent surrogate marker for MDR-TB. I’m sure Koch and Pasteur would not only be delighted with the technological advance, they would probably say, **‘It’s about time’.**”

Fred Tenover, Ph.D.
Cepheid Senior Director of Scientific Affairs



The Need

Current Testing Methodologies Are Too Slow

According to the World Health Organization (WHO), *Mycobacterium tuberculosis* (MTB) is considered to be vastly under diagnosed today, despite approximately 500,000 new active cases reported in the WHO European region during 2007. This is a direct result of current MTB testing methods requiring weeks to deliver a definitive result, which can lead to patients being left untreated or placed on ineffective therapies. These patients may continue to spread MTB to others in the community, increasing the disease burden.

Drug Resistant Strains

With the worldwide re-emergence of TB, multi-drug resistant (MDR) and extensively drug resistant (XDR) strains have become an even greater threat. According to the WHO Global Tuberculosis Control Report 2009, there may be more than 500,000 cases of MDR-TB worldwide. Current testing for drug resistance can take more than 4 weeks, leading to higher mortality and the further spread of MDR strains.

The Solution

Xpert[®] MTB/RIF

- Simultaneous detection of both MTB and rifampicin resistance, a marker for MDR strains
- Unprecedented sensitivity for detecting MTB — even in smear negative, culture positive specimens
- Results in two hours; requires no instrumentation other than the GeneXpert[®] System
- On-demand results enable physicians to treat rapidly and effectively

Sensitive

- Hemi-nested PCR increases sensitivity
- Highly sensitive for confirmation of both smear positive and smear negative samples
- Robust sonication/mechanical DNA extraction procedure
- Internal extraction control assures extraction performance

XPRT® MTB/RIF ASSAY VERSUS AFB AND CULTURE STATUS

		AFB-		AFB+
		Culture POSITIVE	Culture NEGATIVE	Culture POSITIVE
Xpert MTB/RIF	MTB DETECTED	70	3	275
	MTB NOT DETECTED	7	171	0

SENSITIVITY: **98.0%**
SPECIFICITY: **98.3%***

* The sensitivity of the Xpert MTB/RIF assay in patient samples classified as smear negative, culture positive (S-C+) is 90.9% (70/77) and 100% (275/275) for those classified (S+C+).

Specific

- The Xpert MTB/RIF assay uses 3 specific primers and 5 unique molecular probes to ensure a high degree of specificity
- Assay targets the *rpoB* gene, which is critical for identifying mutations associated with rifampicin resistance
- No cross reactions were observed with many other bacterial species tested, including a comprehensive panel of Mycobacteria

rpoB GENE 81 bp RIF RESISTANCE DETERMINING REGION



Efficient

- Rapid, on-demand results mean infected patients can be placed in isolation efficiently and treated with the appropriate therapeutics effectively
- *Mycobacterium tuberculosis* — treat and isolate
- Rapid detection of rifampicin-resistance not only guides individual patient therapy but aids with infection control and public health activities as well

PERFORMANCE CHARACTERISTICS OF THE XPRT MTB/RIF ASSAY COMPARED TO DRUG SUSCEPTIBILITY TESTING FOR RIFAMPICIN (RIF)

		DST	
		RIF Resistant	RIF Sensitive
Xpert MTB/RIF	RIF Resistance DETECTED	58	4
	RIF Resistance NOT DETECTED	2	280

SENSITIVITY: **96.7%**
SPECIFICITY: **98.6%**
PPV: **93.6%**
NPV: **99.3%**

Advantage: Test and Treat

Xpert® MTB/RIF

- Results in 2 hours, available on-demand
- *M. tuberculosis* detected/not detected
- Rifampicin resistance

AFB Smear Test

- Results in 2–24 hours
- Acid fast bacilli present/absent

Culture Testing Results: 2–4 Weeks

- MTB detected/not detected

Testing for Drug Resistance Results: 2–4 Weeks

- Resistance detected/not detected

Workflow: Self contained cartridge – just add sample

1 Pour Sample Reagent into sample tube.

Incubate for 15 minutes at room temperature.
(Acceptable sample types: unprocessed sputum or sediment from concentrated specimen.)



1

2 Pipette diluted sample into cartridge.



2

3 Insert cartridge and start assay.



3

TOTAL HANDS-ON TIME = 2 MINUTES

ORDERING INFORMATION

Xpert® MTB/RIF (10 Cartridges with Sample Reagent) Catalog No. GXMTB/RIF-10

References:

1. Reducing the Global Burden of Tuberculosis: The Contribution of Improved Diagnostics. *Nature* 444 (2006): 49–57
2. Hernández-Garduño E, Cook V, Kunimoto D, Elwood RK, Black WA, Fitzgerald JM: Transmission of tuberculosis from smear negative patients: a molecular Epidemiology study. *Thorax* 2004, 59:286–290
3. National Institute for Clinical Excellence (NICE) Clinical Guideline 33, 2006

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GeneXpert.
Powered By CEPHEID INNOVATION

Xpert[®] MTB/RIF

REF CGXMTB/RIF-10

Authorized for use ONLY in FIND Target Populations in Designated Countries



In Vitro Diagnostic Medical Device



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English

In Vitro Diagnostic Medical Device

Proprietary Name

Xpert® MTB/RIF

Common or Usual Name

MTB/RIF Assay

Intended Use

The Xpert MTB/RIF test for use with the Cepheid GeneXpert® System is a semi-quantitative nested real-time PCR *in-vitro* diagnostic test for: 1) the detection of *Mycobacterium tuberculosis* complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative; and 2) the detection of rifampin-resistance associated mutations of the *rpoB* gene in samples from patients at risk for rifampin resistance.

The MTB/RIF test is intended for use with specimens from untreated patients for whom there is clinical suspicion of tuberculosis (TB). Use of Xpert MTB/RIF for detection of *M. tuberculosis* (MTB) or determination of rifampin susceptibility has not been validated for patients who are receiving treatment for tuberculosis.

Summary and Explanation

Globally, around 2 billion people are infected with *M. tuberculosis*¹. Every year almost 9 million people develop active disease and 2 million people lose their lives to the illness. Active tuberculosis is predominantly pulmonary in nature. 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), Rifampicin (also known as Rifampin, RIF), Ethambutol (EMB), and Pyrazinimide (PZA). As documented by WHO, RIF resistance is rarely encountered by itself, and usually indicates resistance to a number of other anti-TB drugs². It is most commonly seen in multi-drug resistant (MDR-TB) strains and has a reported frequency of greater than 95% in such isolates^{3, 4, 5}. MDR-TB is defined as a tuberculous disease caused by a bacterial strain that is resistant to at least INH and RIF. 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 speeds the diagnosis of both drug-susceptible and MDR tuberculosis. With the Xpert MTB/RIF test, this can be accomplished in fresh sputum samples and in prepared sediments in less than 2 hours. The rapid detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy during the same medical encounter.


Principle of the Procedure

The GeneXpert Dx System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected 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 eliminated. For a full description of the system, see the *GeneXpert Dx System Operator Manual*.

Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) 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 in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair “core” region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

Reagents and Instruments

 **Material Provided**
The Xpert MTB/RIF kit (CGXMTB/RIF-10) contains sufficient reagents to process 10 patient or quality-control specimens.

The kit contains the following:

Xpert MTB/RIF cartridges with integrated reaction tubes	10
Bead 1 (freeze-dried)	2 per cartridge
<ul style="list-style-type: none"> • Primers • Probes • KCl • MgCl₂ • HEPES, pH 8.0 • BSA (bovine serum albumin) 	
Bead 2 (freeze-dried)	2 per cartridge
<ul style="list-style-type: none"> • Polymerase • KCl • MgCl₂ • dNTPs • HEPES, pH 7.2 • BSA (bovine serum albumin) 	
Bead 3 (freeze-dried)	1 per cartridge
<ul style="list-style-type: none"> • Sample Processing Control (SPC) ~2000 non-infectious sample preparation control spores 	
Reagent 1 (Tris Buffer, EDTA, and surfactants)	4 mL per cartridge
Reagent 2 (Tris Buffer, EDTA, and surfactants)	4 mL per cartridge
Sample Reagent (Sodium Hydroxide and Isopropanol)	10 x 8 mL bottles
Sterile disposable transfer pipettes	12

Note:

- The Sample Reagent solution is clear, ranging from colorless to golden yellow.
- Material Safety Data Sheets (MSDS) for all reagents provided in this assay are available upon request from Cepheid Technical Support and the Cepheid website www.cepheid.com.
- The bovine serum albumin (BSA) in this product was produced 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 commingling of the material with other animal materials.
- The sterile transfer pipettes have a single mark representing the minimum volume of sample necessary to transfer to the GX cartridge. Use only for this purpose. All other sterile pipettes must be provided by the laboratory.

Storage and Handling



- Store the Xpert MTB/RIF cartridges and reagents at 2–28 °C.




- Do not use reagents or cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge within 30 minutes after opening the cartridge lid.
- The cartridge is stable up to 7 days after opening the package.

Materials Required but Not Provided

- GeneXpert Dx System equipped with GX2.1 software (catalog number varies by configuration): GeneXpert instrument, computer, barcode wand reader, and Operator Manual
- Printer (See *GeneXpert Dx System Operator Manual* for compatibility guidelines)
- Sterile screw-capped specimen collection containers
- Disposable Gloves
- Labels and/or indelible labeling marker
- Sterile pipettes for sample processing

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 universal precautions.
 - Guidelines for specimen handling are available from the U.S. Center for Disease Control and Prevention⁶ and the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).⁷
 - Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents. Follow your institution's safety procedures for working with chemicals and handling biological samples.
 - The performance of Xpert MTB/RIF for the detection of MTB complex has not been demonstrated from non-respiratory specimens such as blood, CSF, stool or urine. The performance of the Xpert MTB/RIF test has not been evaluated with specimens processed by methods other than those described in this package insert.
 - Do not open the Xpert MTB/RIF cartridge lid except when adding sample.
 - Do not use a cartridge that has been dropped or shaken after you have added the treated sample.
 - 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.
-  Each single-use Xpert MTB/RIF cartridge is used to process one test. Do not reuse spent cartridges.
- Dispose of used Xpert MTB/RIF cartridges according to your institution's and country's safety guidelines for hazardous material.



Specimen Collection and Transport

To obtain adequate specimen, follow the instructions in this section closely.

Note: Collect a minimum of 1 mL of sputum per specimen. Subject must be seated or standing.

Specimens should be held at 2–8 °C prior to processing whenever possible. However, if necessary the specimens can be stored at a maximum of 35 °C for ≤ 3 days and at 4 °C for days 4–10.

1. Rinse the patient's mouth twice with water.
2. Unscrew the lid on the sputum collection container.
3. Have the patient inhale deeply, cough vigorously, and expectorate the material into the sterile screw-capped specimen collection container. Avoid spills or soiling the outside of the container
4. Secure the lid on the collection device.
5. Specimens should be held at 2–8 °C whenever possible including during transport to the laboratory.

Procedure – Sputum Sediments

Note: Do not accept specimens with obvious food particles or other solid particulates.

Note: Process only as many samples at one time as there are modules available to run the test on the GeneXpert Dx System. Guidelines for handling TB should be closely followed.

Volume Requirements—Sputum sediments prepared according to the method of Kent and Kubica⁸ and re-suspended in 67mM Phosphate/H₂O buffer) can be tested using Xpert MTB/RIF. Once the resuspension is prepared for standard laboratory smear or culture tests, ensure at least 0.5 mL of resuspended sediment is available to run Xpert MTB/RIF.

1. Label each Xpert MTB/RIF cartridge with the sample ID. (Write on the sides of the cartridge or affix ID label.) Note: Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge.

2. Transfer at least 0.5 mL of the total resuspension pellet to a conical, screw-capped tube for the Xpert MTB/RIF using a sterile transfer pipette. Alternatively, the entire sample may be processed in the original tube.
3. Store re-suspended sediments at 2–8 °C if they are not immediately processed for Xpert MTB/RIF. Do not store for more than 12 hours.
4. Add 1.5 mL of Xpert MTB/RIF Sample Reagent (SR) to 0.5 mL of resuspended sediment sample using a sterile transfer pipette and shake vigorously 10 – 20 times. Note: One back-and-forth movement is a single shake.
5. Incubate the specimen for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation, again shake the specimen vigorously 10 – 20 times. Samples should be liquefied with no visible clumps of sputum. Particulate matter may exist that is not part of the sample.

Procedure – Expecterated Sputum Samples

Note: Do not accept specimens with obvious food particles or other solid particulates.

Note: Process only as many samples at one time as there are modules available to run the test on the GeneXpert Dx System. Guidelines for handling TB should be closely followed⁶.

1. Label each Xpert MTB/RIF cartridge with the sample ID. (Write on the sides of the cartridge or affix ID label.) Note: Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge.
2. Leave specimen in leak-proof sputum collection container.
3. For each of the samples; unscrew lid of sputum collection container; add Sample Reagent 2:1 (v/v) to sample, replace the lid, and shake vigorously 10 - 20 times. Note: One back-and-forth movement is a single shake.
4. Incubate for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation again shake the specimen vigorously 10 – 20 times. Samples should be liquefied with no visible clumps of sputum. Particulate matter may exist that is not part of the sample.

Procedure

Preparing the Cartridge

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

1. Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. Do not process the sample further if there is insufficient volume.
2. Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge. See Fig. 1, below. Dispense slowly to minimize the risk of aerosol formation.
3. Close the cartridge lid. Make sure the lid snaps firmly into place. Remaining liquefied sample may be kept for up to 12 hours at 2 – 8 °C should repeat testing be required.

Important: Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of preparing the cartridge.

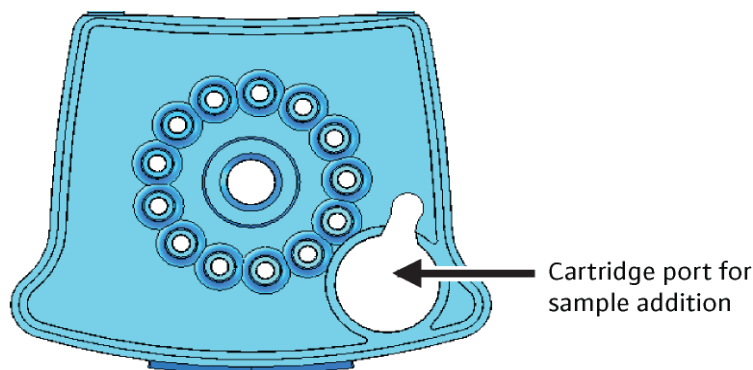


Figure 1. Xpert MTB/RIF cartridge (top view).

Starting the Test

Important: Before you start the test, ensure that the system is equipped with the GX2.1 software, and the Xpert MTB/RIF assay 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*.

1. Turn on the computer, and then turn on the GeneXpert Dx instrument.
2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
3. Log on to the GeneXpert Dx System software using your user name and password.
4. In the GeneXpert Dx System window, click **Create Test**. The Scan Cartridge Barcode dialog box appears.
5. Scan the barcode on the Xpert MTB/RIF cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
6. In the **Sample ID** box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the “**View Results**” window and all the reports.
7. Click Start Test. In the dialog box that appears, type your password.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.
11. Dispose of used cartridges in the appropriate specimen waste containers according to your institution’s standard practices.

Viewing and Printing Results

For detailed instructions on how to view and print the results, see the Cepheid *GeneXpert Dx System Operator Manual*.

CONTROL

Quality Control

Each test includes a Sample Processing Control (SPC) and probe check (PCC).

Sample Processing Control (SPC)—Ensures 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.

Probe Check Control (PCC)—Before the start of the PCR reaction, the GeneXpert Dx 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.



Figure 2. GeneXpert Dx System—Privileged User View Results window, MTB Detected Low, Rif Resistance DETECTED



Figure 3. GeneXpert DX System—Privileged User View Results window, MTB Detected Medium, Rif Resistance NOT DETECTED



Figure 4. GeneXpert Dx System—Privileged User View Results window, MTB NOT DETECTED

Interpretation of Results

The results are interpreted by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and will be displayed in the “View Results” window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.

MTB Detected

MTB target DNA is detected.

- MTB Detected—The MTB result will be displayed as High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample. Table 1 lists the Ct value ranges for the displayed MTB results.

Table 1. MTB result name and C_t value range

MTB result	C_t range
High	<16
Medium	16–22
Low	22–28
Very Low	>28

- Rif Resistance DETECTED, Rif Resistance NOT DETECTED, or Rif Resistance INDETERMINATE will be displayed only in MTB DETECTED results and will be on a separate line from the MTB DETECTED result.
- Rif Resistance DETECTED; a mutation in the *rpoB* gene has been detected that falls within the valid delta Ct setting.
- Rif Resistance INDETERMINATE; the MTB concentration was very low and resistance could not be determined.
- Rif Resistance NOT DETECTED; no mutation in the *rpoB* gene has been detected.
- SPC—NA (not applicable); SPC signal is not required since MTB amplification may complete with this control.
- Probe Check—PASS; all probe check results pass.

MTB Not Detected

MTB target DNA is not detected, SPC meets acceptance criteria.

- MTB NOT DETECTED—MTB target DNA is not detected
- SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.
- Probe Check—PASS; all probe check results pass.

RIF Not Detected

RIF target DNA is not detected, SPC meets acceptance criteria.

- RIF NOT DETECTED—RIF target DNA is not detected
- SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.
- Probe Check—PASS; all probe check results pass.

INVALID

Presence or absence of MTB cannot be determined, repeat test with extra specimen. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

- MTB INVALID—Presence or absence of MTB DNA cannot be determined.
- SPC—FAIL; MTB target result is negative and the SPC Ct is not within valid range.
- Probe Check—PASS; all probe check results pass.

ERROR

- MTB—NO RESULT
- SPC—NO RESULT
- Probe Check—FAIL*; one or more of the probe check results fail.

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

NO RESULT

- MTB—NO RESULT
- SPC—NO RESULT
- Probe Check—NA (not applicable)

Reasons to Repeat the Assay

Repeat the test using a new cartridge or initiate alternate procedures if one of the following test results occurs:

- 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 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 there was a GeneXpert module failure.
- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Limitations

The performance of the Xpert MTB/RIF was validated using the procedures provided in this package insert. Modifications to these procedures may alter the performance of the test. Results from the Xpert MTB/RIF should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Because the detection of MTB is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage. Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection procedure, 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 Rifampicin resistance.

Test results might be affected by antecedent or concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.

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

Performance Characteristics

Clinical Evaluation Study Design

Performance characteristics of the Xpert MTB/RIF were determined at two geographically diverse clinical sites by comparing Xpert MTB/RIF on the GeneXpert System with 1) Acid Fast Bacillus (AFB) smear microscopy, 2) liquid and solid culture (Becton Dickinson BACTEC™X MGIT™, Lowenstein-Jensen), and 3) drug susceptibility testing (DST) [covering drugs INH, RIF, EMB, SM (Streptomycin), and PZA]. Site 1 is located in a resource-poor country in an urban area with a high TB case notification rate (192 per 100,000 inhabitants) and is enrolling patients from surrounding treatment centers after a clinical screening for TB. The smear positivity rate in this setting is therefore approximately 62%. Site 2 is located in a high MDR-TB prevalence country. The majority of study participants were MDR suspects with a smear positivity rate of about 30%.

The proportion of patients with a prior history of TB is approximately 24% and 56% for sites 1 and 2, respectively. At both sites, the HIV co-infection rate is below 10%. Laboratories providing the smear microscopy and solid and liquid culture results are quality-assured reference laboratories. Whereas the laboratory for site 1 was located next to the clinic, sputum samples at site 2 were shipped (at 4 °C) to the reference laboratory for testing. The study was performed prospectively with individuals suspected of having clinical pulmonary TB with persistent cough for two weeks or greater. Each enrolled individual provided 3 sputum samples of sufficient quantity within 72 hours at Site 1 and 168 hours at Site 2. Two of the three sputum samples for each patient (minimum 1.5 mL) were processed using NALC (n-acetylcysteine)/NaOH decontamination, and tested using smear microscopy, culture, DST^{9,10,11} and Xpert MTB/RIF (smear and Xpert MTB/RIF were done from the same sediment as culture). The third sputum was tested by direct smear and Xpert MTB/RIF. Drug susceptibility testing was carried out for the first positive culture of each specimen.

Overall Results

A total of 1697 specimens from 526 patients were tested for *M. tuberculosis* and RIF resistance at the two sites and compared to AFB smear and culture.

The smear and culture patient results tabulated below are aggregate results for all specimens for each patient compared to the aggregate Xpert MTB/RIF results for each patient. A smear-negative result is defined as all negative smears or at most 1 scanty smear and the rest negative. A smear-positive result is a single positive smear of 1+ or greater or 2 scanty smears. A patient is culture-positive if a single culture is positive and an Xpert MTB/RIF result for a given patient is positive if a single result is positive.

Xpert MTB/RIF versus Culture Status

Table 2 summarizes the Xpert MTB/RIF result versus the final culture result stratified by the specimen smear status. A patient is defined as MTB culture positive if at least 1 of the culture results is positive, whereas a culture negative patient is defined as all culture results are negative.

Table 2. Xpert MTB/RIF v. AFB and Culture Status

		Smear Negative (AFB-)		Smear Positive (AFB+)			
		Culture Positive	Culture Negative	Culture Positive			
Site 1	Xpert MTB/RIF	MTB Detected	10	0	199	PPV	100%
		MTB Not Detected	2	102	0	NPV	98.1%
		Sensitivity		Specificity			
		99.1%		100%			

		AFB-		AFB+			
		Culture Positive	Culture Negative	Culture Positive			
Site 2	Xpert MTB/RIF	MTB Detected	60	3	76	PPV	97.8%
		MTB Not Detected	5	69	0	NPV	93.2%
		Sensitivity		Specificity			
		96.5%		95.8%			

		AFB-		AFB+			
		Culture Positive	Culture Negative	Culture Positive			
Combined	Xpert MTB/RIF	MTB Detected	70	3	275	PPV	99.1%
		MTB Not Detected	7	171	0	NPV	96.1%
		Sensitivity		Specificity			
		98.0%		98.3%			

The sensitivity of the Xpert MTB/RIF assay in patients classified as smear negative, culture positive (S-C+) is 90.9% (70/77) and 100% (275/275) for those classified as smear positive, culture positive.

The specificity in smear negative, culture negative patients is 100% at Site 1 and 95.8% at Site 2. The combined value is 98.3%.

Table 3. Performance Characteristics of Xpert MTB/RIF compared to Drug Susceptibility Testing for Rifampicin (RIF)

		DST				
		RIF Resistant	RIF Sensitive			
Site 1	Xpert MTB/RIF	RIF Resistance Detected	16	3	PPV	84.2%
		RIF Resistance Not Detected	0	190	NPV	100%
		Sensitivity	Specificity			
		100%	98.4%			

		DST				
		RIF Resistant	RIF Sensitive			
Site 2	Xpert MTB/RIF	RIF Resistance Detected	42	1	PPV	97.7%
		RIF Resistance Not Detected	2	90	NPV	97.8%
		Sensitivity	Specificity			
		95.5%	98.9%			

		DST				
		RIF Resistant	RIF Sensitive			
Combined	Xpert MTB/RIF	RIF Resistance Detected	58	4	PPV	93.6%
		RIF Resistance Not Detected	2	280	NPV	99.3%
		Sensitivity	Specificity			
		96.7%	98.6%			

The Xpert MTB/RIF sensitivity observed for rifampicin resistance is 96.7% and the specificity is 98.6% (rifampicin susceptible).

Interfering Substances

A study was performed to assess the potential inhibitory effects of substances that may be present in sputum processed with the Xpert MTB/RIF assay. These include, but are not limited to: blood, pus, mammalian cells and hemoglobin. These substances were tested at 5% final sample concentration (blood, pus, mammalian cells) or 0.2% (hemoglobin) on the performance of the Xpert MTB/RIF. Each substance was added to a sample containing approximately 5 x limit of detection (LoD) of target BCG cells and tested in duplicate.

No inhibitory effect was observed for any of the above potentially interfering substances.

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* cells spiked into negative clinical sputum samples. Under the conditions of the study, results indicate that the LoD point estimate for *M. tuberculosis* is 131 CFU/mL with a 95% confidence interval ranging from 106.2 CFU to 176.4 CFU. The estimate and confidence levels were determined using logistic regression with data (number of positives per number of tests at each level) taken at different concentrations.

The confidence intervals were determined using the maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix.

Analytical Specificity (Exclusivity)

Cultures of 18 nontuberculosis mycobacteria, NTM (formerly MOTT), strains were tested with the Xpert MTB/RIF assay. Two or more replicates of each isolate were spiked into negative sputum samples and tested at a concentration of 10^6 CFU/mL.

Table 4. NTM strains tested for specificity

NTM Strains Tested (10^6 cfu/mL)			
1	<i>M. avium</i> , SmT Mc2, 2500	10	<i>M. genevenses</i> , #51233
2	<i>M. avium</i> , SmD Mc2, 2501	11	<i>M. xenopi</i> , #2278
3	<i>M. intracellular</i> , #35790	12	<i>M. szulgai</i> , Cap E9-1997
4	<i>M. intracellular</i> , #35776	13	<i>M. celatum</i> , #51131
5	<i>M. kansasii</i> , #35776	14	<i>M. marinum</i> , Cap E10
6	<i>M. scrofulaceum</i> , Cap E5-1985	15	<i>M. simiae</i> , #25275
7	<i>M. malmoense</i> , #29571	16	<i>M. asiaticum</i> , E1-1985
8	<i>M. fortuitum</i> , #35754	17	<i>M. thermoresistable</i> , e22-1985
9	<i>M. chelonae</i> , #35749	18	<i>M. flavescens</i> , PoH 193D

Under the conditions of the study, all of the NTM isolates were reported MTB negative.

Additionally, in order to determine if high concentrations of NTM would interfere with the detection of low levels of TB, the strains listed in Table 5 were mixed with the TB strain H37Rv in sputum to a final concentration of 10^6 cfu/mL NTM and 200 cfu/mL H37Rv.

Table 5. NTM strains tested for ability to interfere with TB detection

Strains Tested
<i>M. avium</i> , SmT Mc2, 2500
<i>M. avium</i> , SmD Mc2, 2501
<i>M. intracellular</i> , #35790
<i>M. intracellular</i> , #35776
<i>M. kansasii</i> , #35776
<i>M. malmoense</i> , #29571

Five of the six strains did not interfere in the detection of 200 cfu/mL of *M. tuberculosis*; thus, the signals were the same as H37Rv alone. The sixth, *M. malmoense*, produced a weak interference at 10^6 cfu/mL but none at 10^5 cfu/mL or lower. Therefore, there is no interference in the detection of *M. tuberculosis* even with 10^5 cfu/mL of NTM.

Non-mycobacterial organisms (n = 61) that represent a wide-range of pathogens, common contaminants and microflora commonly present in sputum or the mouth were tested at a concentration of 10^6 copies per final reaction volume. All organisms were correctly identified as MTB-negative by the Xpert MTB/RIF assay. Positive and negative controls were included in the study. The specificity was 100%.

Species/Strains tested for Specificity

Table 6. Species/Strains tested for specificity

<i>Acinetobacter baumannii</i>	<i>Haemophilus parahemolyticus</i>	<i>Shigella boydii</i>
<i>Acinetobacter calcoaceticus</i>	<i>Haemophilus parainfluenzae</i>	<i>Shigella flexneri</i>
<i>Actinomyces meyeri</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>
<i>Bacillus cereus</i>	<i>Legionella pneumophila</i>	<i>Staphylococcus capitis</i>
<i>Bacillus subtilis</i>	<i>Leuconostoc mesenteroides</i>	<i>Staphylococcus epidermidis</i>
<i>Bordetella parapertussis</i>	<i>Listeria grayi</i>	<i>Staphylococcus haemolyticus</i>
<i>Campylobacter jejuni</i>	<i>Moraxella catarrhalis</i>	<i>Staphylococcus hominis</i>
<i>Candida albicans</i>	<i>Morganella morganii</i>	<i>Stenotrophomonas maltophilia</i>
<i>Citrobacter freundii</i>	<i>Mycoplasma pneumoniae</i>	<i>Streptococcus equi</i>
<i>Corynebacterium pseudodiphtheriticum</i>	<i>Neisseria gonorrhoeae</i>	<i>Streptococcus pyogenes</i>
<i>Corynebacterium xerosis</i>	<i>Neisseria lactamica</i>	<i>Streptococcus agalactiae</i>
<i>Cryptococcus neoformans</i>	<i>Neisseria meningitidis</i>	<i>Streptococcus constellatus</i>
<i>Enterobacter aerogenes</i>	<i>Neisseria mucosa</i>	<i>Streptococcus mitis</i>
<i>Enterobacter cloacae</i>	<i>Peptostreptococcus anaerobius</i>	<i>Streptococcus mutans</i>
<i>Enterococcus avium</i>	<i>Porphyromonas gingivalis</i>	<i>Streptococcus pneumoniae</i>
<i>Enterococcus faecalis</i>	<i>Prevotella melaninogenica</i>	<i>Streptococcus uberis</i>
<i>Enterococcus faecium</i>	<i>Propionibacterium acnes</i>	<i>Veillonella parvula</i>
<i>Escherichia coli</i> (Strain type 2)	<i>Proteus mirabilis</i>	
<i>Escherichia coli</i> O157H7 (Strain type 1)	<i>Pseudomonas aeruginosa</i>	
<i>Fusobacterium nucleatum</i>	<i>Salmonella typhi</i>	
<i>Haemophilus influenzae</i>	<i>Serratia marcescens</i>	

Analytical Inclusivity

DNA samples from a total of 79 MTB strains were tested on the GX using an Xpert MTB/RIF protocol modified for DNA testing. The final reaction components and PCR cycling conditions were unchanged from the protocol designed for patient sample testing. Seventy of the strains were from the WHO/TDR collection and 9 from the laboratory collection at the University of Medicine and Dentistry of New Jersey (UMDNJ). Collectively these strains represent isolates from 31 countries and contained 37 rifampicin-resistant isolates comprised of 13 unique *rpoB* core region mutations. These include every unique *rpoB* mutation found in the TDR database. The negative reactions used water as sample.

The final reaction mixture contained 90 genomic copies of the isolates in 100 µL total volume.

As shown in the table below, the Xpert MTB/RIF correctly detected all MTB strains and correctly identified the rifampicin resistant isolates.

			GeneXpert Result		
			MTB Positive		MTB Negative
			RIF detected	RIF not detected	
Reference	MTB +	RIF Resistance	37	0	0
		RIF Sensitive	0	42	0
	MTB –		0	0	52

Analytical Inactivation of Mycobacteria in sputum samples

The disinfection capability of the Xpert MTB/RIF sample reagent was determined using a standardized quantitative tuberculocidal culture method. Samples of sputum were spiked with a high concentration of viable *M. bovis*, mixed with sample reagent at a ratio of 2:1, and incubated for 15 minutes. Following incubation the sample reagent/sputum mixture was neutralized by dilution and filtration and then cultured. The viability of the *M. bovis* organisms from the treated sputum was reduced by at least 6 logs relative to the untreated control.

Each laboratory must determine the effectiveness of the sample reagent disinfection properties using their own standardized methods and must adhere to recommended biosafety regulations.

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Assistance

For assistance, contact Cepheid using one of the following contact details. Make sure you provide the instrument serial number and reagent lot ID when you call or email.

North America

For technical support, use the following contact details:

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Email: techsupport@cepheid.com

You can reach Cepheid Technical Support by telephone Monday through Friday, from 6 A.M. to 5 P.M. Pacific Time.

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

Tel: +33.563.82.53.19

Email: techsupport@cepheideurope.fr

Other Locations

Contact your local Cepheid representative.

Table of Symbols

Symbol	Meaning
	Catalog number
	<i>In vitro</i> diagnostic medical device
	Do not reuse
	Caution, consult accompanying document
	Manufacturer
	Contains sufficient for <n> tests
	Expiration date
	Control
	Authorized representative in the European Community
	Temperature limitation
	Biological hazard

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