Mobile X-ray Unit:

GeneXpert MTB/RIF *M. tuberculosis* Assay

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Document Review History

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1	New procedure introduced as POCT on the MXU	R Shorten 31.1.12	S Rattenbury 6.2.12	8.2.12
2	Review. Removal of maintenance procedures. Addition of Royal Free microbiology and Cepheid contact details.	R Shorten 19.03.2014	S Rattenbury 19.03.2014	

Record of Amendments

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0 Introduction

0.1 Purpose and Scope

This procedure describes the molecular detection of *Mycobacterium tuberculosis* and rifampicin resistance in clinical samples using the Cepheid GeneXpert MTB/RIF quantitative polymerase chain reaction (Q-PCR) assay. This assay is performed on the Mobile X-ray Unit (MXU) as a Point of Care Test (POCT).

This procedure is for use in the MXU and only those who have undergone appropriate training and competence assessment may perform this assay.

0.2 Responsibility, Personnel and Competence Assessment

The MXU management team, in collaboration with the Royal Free Hospital Microbiology Laboratory Management Team is responsible for ensuring the implementation and maintenance of this procedure. The procedure has been approved by the Royal Free London NHS Foundation Trust POCT Committee.

This procedure may be performed by fully trained individuals or those undergoing training under supervision.

The MXU Operations Manager (MXU OM) is responsible for overseeing this assay on a day to day basis. All users must raise issues with the MXU OM when appropriate. The MXU OM may consult with the Royal Free Hospital Microbiology Laboratory Management Team as required.

Each individual undertaking this procedure is responsible under Clinical Governance & Health and Safety at Work Act for the Quality of work performed and the safety of themselves and others. Each individual must have their competence assessed by a trained and competent person using a Competence Assessment Form for this procedure (MIC-CAF-GXpert). The user must also read all relevant SOPs and Risk Assessments.

0.3 Principle/Overview of the Procedure

Sputum is expectorated by the patient into a specially designed specimen container. The patient returns the sealed container to the MXU where Sample Reagent is safely added via the rubber septum in the lid of the container. This initial stage causes a reduction in mycobacterial numbers by the order of six logs. This mixture is added to a test cartridge, barcode scanned into the GeneXpert and analysed for approximately 1 hour 45 minutes.

The assay extracts bacterial DNA ultrasonically and performs the real-time amplification and detection in a single cartridge simultaneously. The system targets the *rpo*B gene of the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum* and *M. microti*). Real-time detection is made possible through the use of specifically designed molecular beacons, which fluoresce upon specific binding to the target. Five differently labelled beacons are utilised to detect common single nucleotide polymorphisms (SNPs) that confer resistance to rifampicin (a marker of multi-drug resistant TB (MDRTB)). Amplification of all five targets indicates the presence of *M. tuberculsis* without the common rifampicin resistance-causing SNPs. If one target fails to amplify, resistance is suspected. Each reaction contains amplification Internal Control that is amplified and detected at the same time and in the same reaction. The purpose of this control is to identify potential inhibition from the processed specimen.

0.4 Selection and Validation of process

The following paper analysed samples from over 1700 patients.

Boehme *et al*. N Engl J Med. 2010 Sep 9;363(11):1005-1015.
Rapid Molecular Detection of Tuberculosis and Rifampin Resistance.

Abstract (excerpts)
Samples from patients with suspected TB were assayed for TB and drug resistance with several different techniques. An automated molecular test showed a sensitivity of 98% in smear-positive, culture-positive samples and a sensitivity of 72-90% in smear-negative, culture-positive samples, and it identified rifampin resistance >97% of the time.

Banada *et al*. Journal of Clinical Microbiology, October 2010, p. 3551-3557, Vol. 48, No. 10.
Containment of Bioaerosol Infection Risk by the Xpert MTB/RIF Assay and Its Applicability to Point-of-Care Settings

Abstract
The recently introduced Xpert MTB/RIF assay (Xpert) has point-of-care potential, but its capacity for biohazard containment remained to be studied. We compared the bioaerosols generated by the Xpert assay to acid-fast bacillus (AFB) microscope slide smear preparation. The Xpert assay sample treatment reagent (SR) was also studied for its sterilizing capacity, stability, and effect on assay sensitivity after prolonged treatment. During the preparation of AFB smears, sputum samples spiked with *Mycobacterium bovis* BCG at 5 x 10^8 CFU/ml produced 16 and 325 CFU/m^3 air measured with an Andersen impactor or BioSampler, respectively. In contrast, neither the sample preparation steps for the Xpert assay nor its automated

processing produced any culturable bioaerosols. In testing of SR sterilizing capacity, clinical sputum samples from strongly smear-positive tuberculosis patients treated with SR at a 2:1 ratio eliminated *Mycobacterium tuberculosis* growth in all but 1/39 or 3/45 samples cultured on solid or liquid medium, respectively. These few unsterilized samples had a mean 13.1-day delay in the time to positive culture. SR treatment at a 3:1 ratio eliminated growth in all samples. SR retained a greater than 6-log-unit killing capacity despite storage at temperatures spanning 4 to 45°C for at least 3 months. The effect of prolonged SR sample treatment was also studied. Spiked sputum samples could be incubated in SR for up to 3 days without affecting Xpert sensitivity for *M. tuberculosis* detection and up to 8 h without affecting specificity for rifampin resistance detection. These results suggest that benchtop use of the Xpert MTB/RIF assay limits infection risk to the user.

0.5 References

CPA Standards E, F, G.

Boehme *et al* Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. N Engl J Med. 2010 Sep 9; 363(11):1005-1015

Banada *et al*. Journal of Clinical Microbiology, October 2010, p. 3551-3557, Vol. 48, No. 10. Containment of Bioaerosol Infection Risk by the Xpert MTB/RIF Assay and Its Applicability to Point-of-Care Settings

0.6 Definitions

MXU	Mobile X-ray Unit
OM	Operations Manager
Q-PCR	Quantitative Polymerase Chain Reaction
*rpo*B	RNA polymerase gene. Mutations in this gene can confer resistance to rifampicin.
RRDR	Rifampicin Resistance Determining Region
MDR TB	Multi drug resistant TB – *M. tuberculosis* that is resistant to at least isoniazid and rifampicin.

0.7 Related documents

1. Policy for Health and Safety (MIC.MPOL-H&S-02)
2. Risk Assessment/COSHH – MXU: GeneXpert MTB/RIF *M. tuberculosis* assay (MIC.MF-COSHH 72)
3. UCLH Waste Management Policy (003/FAC/T)

1 Pre-Examination Process

1. Specimen Handling

1.1 Specimen Transportation & Reception

Samples are produced on site by patients who have an abnormal chest x-ray on the MXU. These samples are only to be tested using the MTB/RIF assay and are not to be referred elsewhere for any further analysis.

1.2 Specimen Types, Requirements and Rejection Criteria

Only freshly expectorated sputum is to be tested. Salivary samples must be rejected. The specimen container must be labelled with the patient details.

1.3 Urgent specimens

All samples are considered urgent and must be processed as soon as possible. This is because the patient may wait in the MXU until the results is available.

1.4 Storage of specimens prior to testing

Samples are not to be stored prior to testing. They must be tested immediately.

1.5 Computer entry of specimen demographics

A patient identifier must be entered onto the GeneXpert software when analysis is to begin – see section 6.

1.6 Referral to other laboratories

Not applicable.

Examination process

2 Health & Safety

Refer to (MIC.MF-COSHH 72) for further information regarding the associated risk assessment and COSHH.

3 Equipment / Materials

	Supplier	Part number
Cepheid GeneXpert		
Sample container with rubber septum in the lid	Greiner bio-one	724300
Plastic pastettes	Alpha Laboratories	LW4635
Discard jars	Microbiology Supply Co.	DL64
Vernagel	Vernacare Ltd	450MA100
Nitrile gloves	UCLH	
Safety goggles	Royal Free Microbiology	

4 Reagents

Cepheid GeneXpert MTB/RIF kit (10)	Cepheid	GXMTB-10

5 Internal Quality Control (IQC)

Each batch of kits is quality controlled by Cepheid. In addition, each cartridge contains a Probe Check Control (PCC). This takes place after sample preparation, bead reconstitution, and tube filling (prior to thermal cycling). Multiple fluorescent readings are taken at different temperatures, which verify the reagent rehydratation, PCR tube filling in the cartridges, probe integrity and dye stability. The probe check values for each test are compared to the value obtained in house for each new lot number. These values are imported in the system automatically when you scan the cartridge.

Each cartridge also contains a Specimen Processing Control (SPC). This is in the form of *Bacillus globidii* spores and control adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction.

6 Procedure

6.1 Specimen processing

NB 1. Up to two samples may be analysed at once.

NB 2. Nitrile gloves and protective spectacles must be worn throughout this procedure

6.1.1 Ensure that the lid of the sample container is secure.

6.1.2 Check the patient details on the side of the container.

6.1.3 Estimate the quality and volume of sputum inside the container (the container is transparent so the lid must not be removed to do this).

6.1.4 Ensure that the power supply is….. Ensure that the GeneXpert is on (there will be a blue light on at the front (centre, bottom) of the instrument.

6.1.5 Using a plastic pipette, add 2-3x of the sputum volume of Sample Reagent via the rubber septum.

NB 3. The end of fine-tipped pipettes must be removed with scissors to enable the passage of reagents and sputum and to reduce aerosol production.

NB 4. The Sample Reagent must be added smoothly down the inside of the container wall. It must not be ejected forcibly onto the sputum as this will create aerosols.

6.1.6 More than one addition of Sample Reagent may be required to ensure the appropriate volume is added. Remove the pipette once all the Sample Reagent has been added and discard the pipette into a discard jar. The Sample Reagent is single-use – discard after use.

6.1.7 Gently swirl the sample container to mix the sputum and Sample Reagent. Set the container aside safely for 7.5 minutes.

6.1.8 Swirl the mixture again to ensure complete homogenisation and set aside for a further 7.5 minutes.

6.1.9 Open one MTB/RIF cartridge per sample and label the **side** with the patient identifiers. **Do not write anywhere near, nor handle the test cuvette at the rear of the cartridge.** Open the top of the MTB/RIF cartridge.

6.1.10 One sample at a time – at the end of the second incubation, use a fresh pipette to aspirate 2mL of the homogenised sample from the specimen container via the rubber septum.

6.1.11 Gently add approximately 2mL of the homogenised sample to the sample hole in the labeled cartridge (figure 1).

NB 5. This must be performed smoothly to prevent aerosol production.

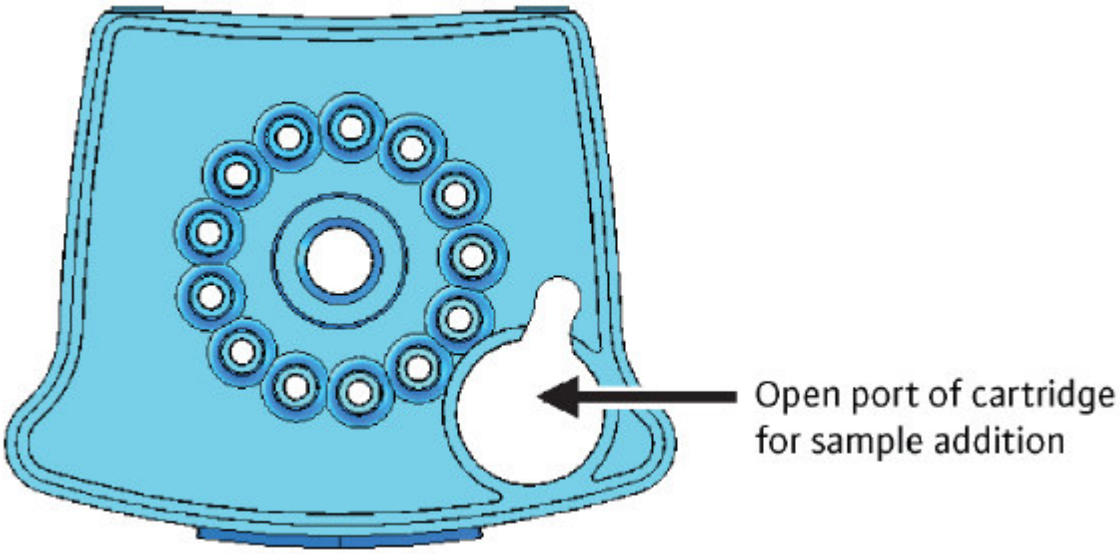

Figure 1. GeneXpert MTB/RIF cartridge (top view).

6.1.12 If ≥2mL of the homogenized sample remains, set this aside safely in case of cartridge failure. If not, discard.the specimen container, pipette and the Sample Reagent vial into the discard jar and close the cartridge lid.

6.1.13 Place the MTB/RIF cartridge(s) **upright** on the bench in front of the GeneXpert.

6.1.14 Ensure that the barcode reader is plugged in and turn on the laptop, Enter the password **cphd** and press enter.

6.1.15 Double click on the GeneXpert DX icon.

6.1.16 Click on Create Test.

6.1.17 Scan the barcode on the front of the cartridge when prompted.

6.1.18 Type in the Patient ID and Sample ID into the appropriate fields.

6.1.19 Click on Start Test.

6.1.20 The GeneXpert will select which module is to be used (A1 or A2). This will be indicated on the screen. If a module is out of use for whatever reason, an alternative may be selected by clicking on the drop-down arrow.

6.1.21 A green light above the selected module will come on. Once the green light starts to flash, pull open the module door, place the cartridge in the module and close the door completely until it clicks. Analysis will now begin and will be complete in approximately 1hour and 45 minutes.

6.1.22 If other samples need to be analysed, repeat steps 6.1.16 onwards.

6.1.23 When analysis is complete, interpret the results on screen as stated in section 8.

6.1.24 Remove the cartridge(s) and place inside a discard jar.

6.2 GeneXpert Maintenance

Three activities are recommended a monthly basis by Cepheid: disinfecting the instrument surfaces, disinfecting the cartridge bay and disinfecting the plunger rod. Due to the low usage of the instrument, these may be performed at the annual service by Cepheid. If any dust, Sample Reagent residue or debris is observed in the cartridge bays, this may be removed with a cotton swab, wetted with clean water.

Caution: Getting liquid inside of the I-CORE module (the hole at the rear of the cartridge bay) can damage the module.

7 Recording of results

Results are recorded in the patient's records.

8 Interpretation of Results

8.1 Repeat criteria and interpretative messages.

8.1.1 Click on View Results.

8.1.2 The result will be displayed in the Test Result field. The result will be listed as one of the following:

MTB NOT DETECTED (figure 2)

MTB DETECTED VERY LOW/LOW/MEDIUM/HIGH
Rif resistance NOT DETECTED (figure 3)

MTB DETECTED VERY LOW/LOW/MEDIUM/HIGH
Rif resistance DETECTED (figure 4)

8.1.3 The positive *M. tuberculosis* DNA result (very low/low/medium/high) is determined by the cycle threshold (CT) value of the assay. The slopes are displayed below the Test Result field. The degree of positivity is not reported - see section 13.

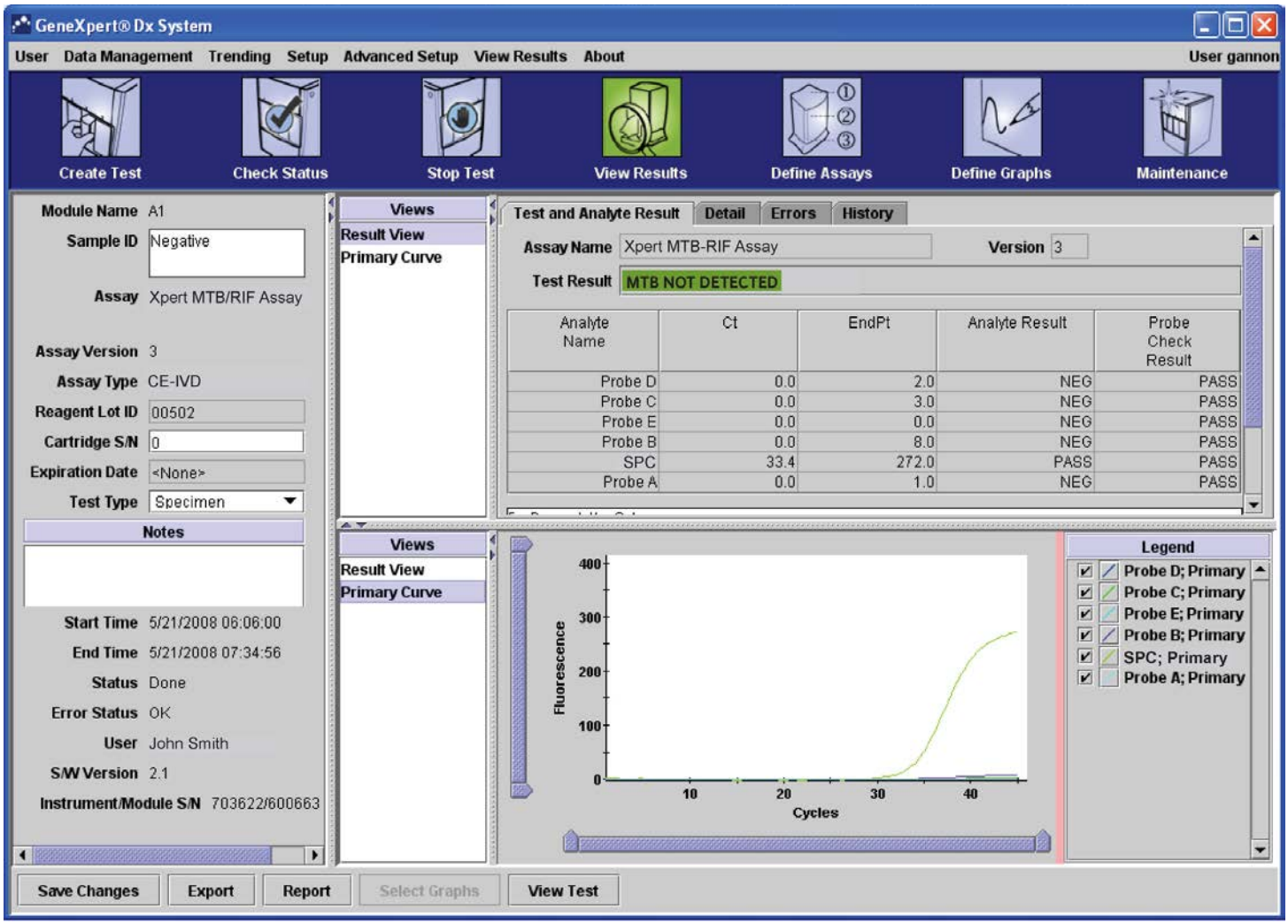

Figure 2. Screen shot of MTB NOT DETECTED

Figure 3. Screen shot of MTB DETECTED. Rif resistance NOT DETECTED

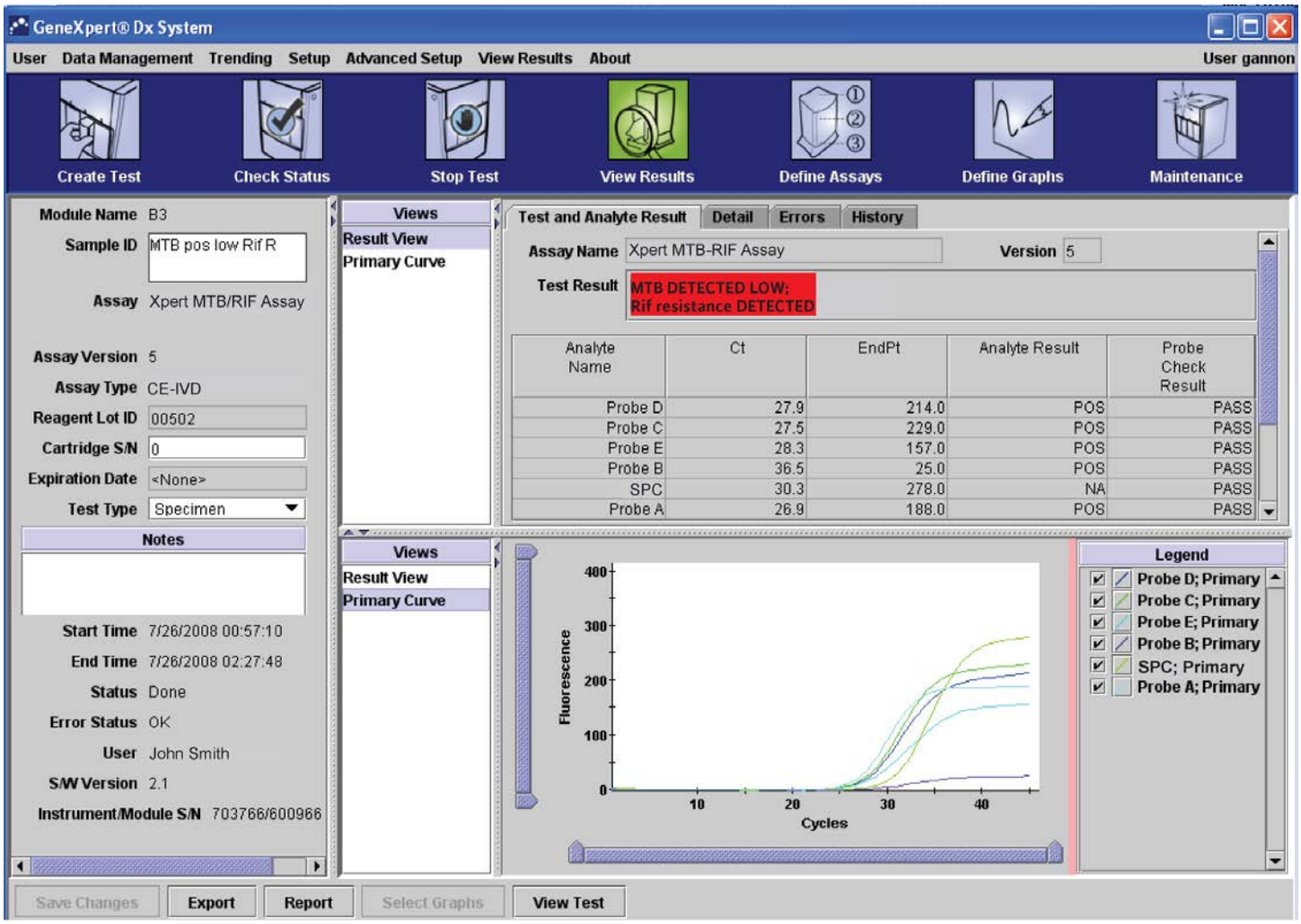

Figure 4. Screen shot of MTB DETECTED. Rif resistance DETECTED

NB 6. If an older result is required, click on the View Results tab on the top toolbar.

8.1.4 A sample may be repeated if any of the following results are obtained:

ERROR
NO RESULT
INVALID

See section 11. Troubleshooting.

If INVALID occurs, this indicates that inhibiting substances are present in the sample.

8.2 Reference ranges.

Not applicable

9 Immediate disposal of material/reagent remains

Plastic pipettes, sample containers, used Sample Reagent and used MTB/RIF cartridges must be placed inside discard jars containing Vernagel. These jars must remain upright at all times when not in use. Each jar must be no more than 75% full.

At the end of the working day, or when the jar is 75% full (whichever is sooner), the jar must be closed (with a lid seal) and placed inside an clinical waste bag. These bags must be placed in the clinical waste stream (for incineration) of the next available NHS institution that the MXU visits.

10 Limitations and pitfalls of the procedure

10.1 This method has only being evaluated for sputum.

10.2 A negative result does not exclude the possibility of the concentration of the target organism being present below the sensitivity of the test.

10.3 As with other diagnostic test, results from this assay should be interpreted in conjunction with other laboratory and clinical data available to the physician.

10.4 The MTB/RIF assay does not differentiate between members of the *M. tuberculosis* complex – *M. tuberculosis, M. bovis, M. bovis* BCG, *M. africanum*, and *M. microti.*

10.5 The assay detects the most commonly occurring mutations in the Rifampicin Resistance Determining Region (RRDR) of the *rpo*B gene. Resistance caused by less common mutations, and those outside of the RRDR will not be detected.

10.6 Optimal performance of this assay requires adequate specimen collection and handling.

10.7 This assay cannot be used to assess therapeutic success or failure since nucleic acids from *M. tuberculosis* complex organisms may persist following antimicrobial therapy.

10.8 The predictive value of the assay depends on the prevalence of the disease in any particular population.

10.9 This assay does not differentiate between live and dead organisms.

10.10 Failure to follow the procedural directions may affect test results.

11 Troubleshooting

In the event of Equipment Error/Fault, record the appropriate details on the 'Equipment Error/Fault Log Form'.

ERROR codes include:

PC values too high (#5006)
PC values too low (#5007)
Max pressure exceeded (#2005)

If ERROR occurs on repeat testing (or other instrument failure occurs), contact Cepheid for advice - **01246 860730**

NO RESULT is triggered by:

MTB - No Result
SPC - No Result
Probe Checks – NA

and may be caused by: the user stopping the run, power failure, or load error

INVALID is triggered when:

Internal control Ct = > 38 or 0
SPC – FAIL
Probe Checks - PASS

This code indicates that the sample contains inhibitors – see sections 8.1 and 13.

12 Validation of Results

Results are only accepted providing the IQC has passed. Results will not be released by the GeneXpert if any controls have failed.

Post-examination process

13 Reporting results

A member of the MXU team, directly or indirectly involved with the process, is responsible for reporting. Results are hand-written onto the existing MXU referral form, which can be reported to the TB team at the hospital to which the patient will be referred.

14 Authorisation of reports / Clinical advice

Authorisation is not required. Clinical advice is available via the UCLH TB team.

15 Storage and disposal of records

Not applicable

16 Storage and disposal of specimen/culture remains

Sample remains and used MTB/RIF cartridges must not be retained. They must be discarded immediately, as stated in section 9.

17 Referral of specimens

Not applicable.

18 Investigation of outstanding results

Not applicable.

19 General housekeeping

Each MXU staff member is responsible for General Housekeeping. The work area where samples are processed and tested must be kept clean and free of clutter at all times.