



Training on Proficiency Testing Scheme (GeneXpert DTS)

Module 11: Panel Validation

Venue
Facilitator's name
Date

Introduction

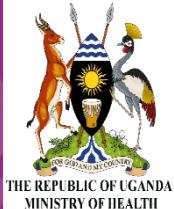
- Panel validation is crucial as a form of confirmation that the panel sent of is table and also provided reference of the expected results.



Objectives

By the end of this module participants should be able to

- Make dilutions and aliquot a PT panel
- Dry a panel at the recommended conditions
- To do a panel validation exercise
- Able to analyze and compile validation data



Course outline

- Panel validation
- Preparation
- Packing and Validation



THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

GXPT/PP/011, Version 2.0, Effective date: 01-Apr-2022

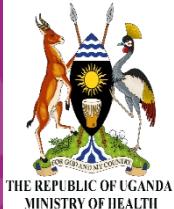
4

UGANDA
Supranational®
Reference Laboratory

Timely Accurate Diagnostics for a TB-Free Africa

Panel selection and Validation

- Ensure the DTS prepared post inactivation are dry
- Test the 5 DTS samples from each of the 8 isolates with Xpert MTB/RIF
- Enter CBNAAT results and Ct data on digital DTS preparation worksheet
- Analyze the standard deviation (SD) and mean of probe A for each stock prepared
- Out of the 8 inactivated stock select 5 for panel preparation
- Vortex with beads, dilute, aliquot, dry, cap, store 2-25°C in the dark



Panel selection and Validation

- Validate panel: test 10% of total samples prepared with CBNAT
- Enter all validation testing results and Ct values into the validation worksheet, analyze SD and mean of probe A to determine if panel passes validation
- Ensure inactivation verification is negative after **84** days
- Label DTS samples, print PT instructions/forms, pack panels and PT packets

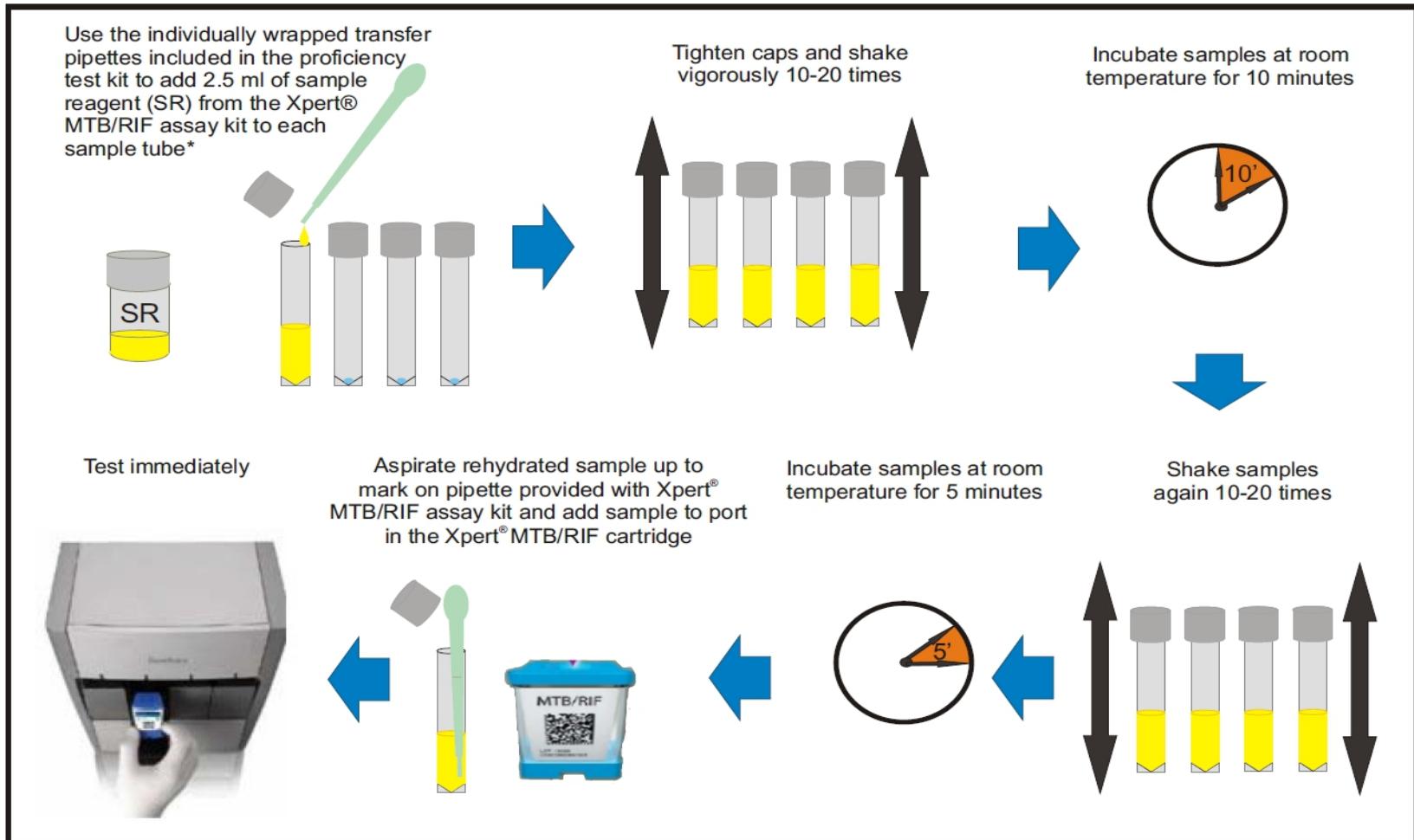


Ship to testing sites or regional labs for distribution

GXPT/PP/011, Version 2.0, Effective date: 01-Apr-2022



Pre-testing with CBNAT



Pre-testing with Enter CBNAI testing data into DTS Preparation Form

CGH/DGHA - International Laboratory Branch - Atlanta, GA

Isolate name _TB35810 Stock # _1602		MTB/RIF Assay Kit Lot #: 09002		Expiry Date: 4/30/2017		Technologist: KD		
Aliquot	Date Tested	Results		Cycle Threshold				
		MTB Detected	Rif Resistance	Probe D	Probe C	Probe E	Probe B	SPC
A	1/13/2016	Medium	Not Detected	20.5	20.3	21.7	20.3	26.9
B	1/13/2016	Medium	Not Detected	19.5	18.3	20.3	18.9	24.2
C	1/14/2016	Medium	Not Detected	21.5	21.5	22.6	21.4	24.7
D	1/14/2016	Low	Not Detected	23.7	23.5	24.6	23.5	27.1
E	1/14/2016	Medium	Not Detected	21.1	20.7	21.9	20.9	25.5
F								
G								
H								
I								
J								
K								
L								
M								
N								
O								
SD				1.558204094	1.888915032	1.570668647	1.682260384	1.2930584
Mean				21.26	20.86	22.22	21	25.68
%CV				7.329276079	9.055201498	7.068715785	8.010763734	5.0352741
Isolate name _H526D Stock # _1603		MTB/RIF Assay Kit Lot #: 09002		Expiry Date: 4/30/2017		Technologist: KD		
Aliquot	Date Tested	Results		Cycle Threshold				
		MTB Detected	Rif Resistance	Probe D	Probe C	Probe E	Probe B	SPC
A	1/14/2016	Low	Detected	0	23.5	24.6	24	25.8
B	1/14/2016	Low	Detected	0	23.3	24.2	23.4	25.6
C	1/14/2016	Low	Detected	0	25.2	26.1	25.5	27.1
D	1/14/2016	Low	Detected	0	24.6	25.7	25.1	26.6
E	1/14/2016	Low	Detected	0	24.5	25.5	24.6	26.1
F								
G								
H								
I								
J								
K								
L								
M								
N								
O								
SD				0	0.798122798	0.791833316	0.84083292	0.6107373
Mean				0	24.22	25.22	24.52	26.24
%CV				#DIV/0!	3.295304697	3.139703869	3.42917178	2.3275048
								3.24374445



Let's look at some pre-test data from a previous validation.....

- Which stocks do you think are the best?
- What resistance pattern do you want your panel to have?



Preparing the final panel

- Work with one stock at a time
- Thoroughly clean the BSC used for aliquoting and drying with 0.5% sodium hypochlorite (10% bleach) followed by 70% ethanol
- Set up the BSC
 - Selected stock from fridge
 - Tubes for aliquots/racks
 - Sterile glass tube with beads
 - Bottle or 50 ml conical for dilution
 - Blue food dye
 - Sterile saline 0.85%
 - Serological pipette and pipette boy or volume metric flasks
 - Repeat pipette and syringe tips
 - Pipette discard



How much do you need to make? How low are your Ct results from pre-test?

1:10 Dilution of stock (use this one if your mean Ct on pretest is below 16)

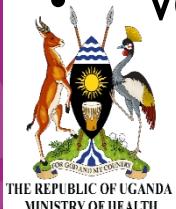
A. Aliquots Per Stock	B. Total Volume 1:10 Diluted Stock Required [(A x 0.1 ml) + 5 ml]	C. Volume Stock Solution Vortexed with Beads [(B/10) + 0.5 ml]	D. Volume Vortexed Stock Added to Saline for 1:10 Dilution (B/10)	E. Volume of saline for 1:10 Dilution (B - D)	Volume Blue Food Grade Dye for 1:1000 Dilution (B/1000)
250	25 ml + 5 ml = 30 ml	3.0 + 0.5 = 3.5 ml	3 ml	27 ml	30 µl
400	40 ml + 5 ml = 45 ml	4.5 + 0.5 = 5.0 ml	4.5 ml	40.5 ml	45 µl

Entire stock plus enough saline to make needed aliquots (use this one for higher Ct on pretest)

A. Aliquots Per Stock	B. Total Volume Diluted Stock Required [(A x 0.1 ml) + 5 ml]	C. Volume Stock Solution	D. Volume of Stock Added to Saline	E. Volume of Saline for Dilution (B - D)	Volume Blue Food Grade Dye for 1:1000 Dilution (B/1000)
250	(250 x 0.1 ml) + 5 ml = 30 ml	Entire volume of stock _____ ml	Entire volume of stock above beads (9 ml)	30 ml - 9 ml = 21 ml	30 ml /1000 = 30µl
500	(500 x 0.1 ml) + 5 ml = 55 ml	Entire volume of stock _____ ml	Entire volume of stock above beads (9 ml)	55 ml - 9 ml = 46 ml	55 ml /1000 = 55µl

Preparing the final panel (cont.)

- Vortex stock for 30 sec
- Transfer stock to tube with beads
- Vortex 5 minutes ensuring a full vortex
- Allow to sit undisturbed for 15 minutes
- Pipette necessary amount of saline and food grade dye into dilution tube
- After 15 minutes has elapsed pipette all the stock from above the beads and transfer to dilution tube
- Vortex the dilution tube for 30 sec



Preparing the final panel (cont.)

- Aliquot 100 µl into tubes
 - Use a repeat pipette with a syringe tip
 - After aspirating dispense 3-4x back into dilution tube (very important!)
 - Use one tip to aliquot all dilutions for stock
 - After finishing a rack hold the rack and visually observe the inside bottom of the tubes to ensure each tube received 100 µl of dilution
 - Set rack aside to dry and continue with the next rack
 - Ensure BSC is left running



Are the DTS Dry?

- Check the DTS daily
- Tap the bottom of the tube on the work surface of the BSC and watch to see if any liquid splashes up
- Tubes are dry when no liquid splashes up and/or the dried sample appears crystalized
- It is important to allow the DTS to dry as completely as possible
 - Water molecules next to DNA molecule will bounce around as they warm up and bump into and harm DNA molecules >>DNA degradation>>False negative

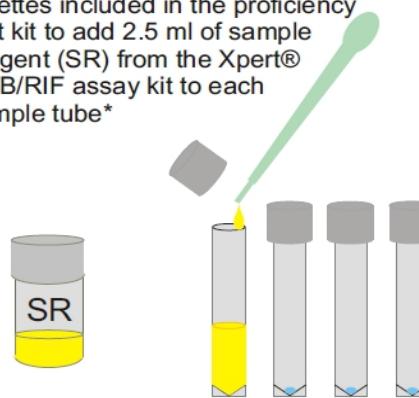
DTS Storage

- DTS can be stored at 2-25°C
- If the A/C is routinely turned off in the evenings or weekends place DTS sample in a container (box) to protect from light and store at 2-8°C

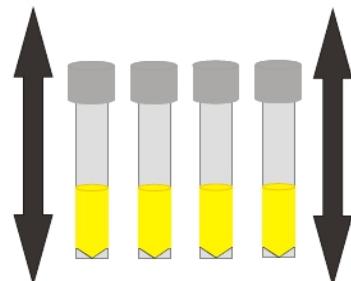


Validate with CBNAAT

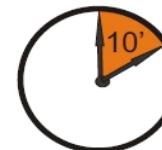
Use the individually wrapped transfer pipettes included in the proficiency test kit to add 2.5 ml of sample reagent (SR) from the Xpert® MTB/RIF assay kit to each sample tube*



Tighten caps and shake vigorously 10-20 times



Incubate samples at room temperature for 10 minutes



Test immediately



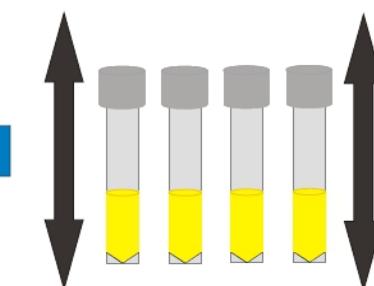
Aspirate rehydrated sample up to mark on pipette provided with Xpert® MTB/RIF assay kit and add sample to port in the Xpert® MTB/RIF cartridge



Incubate samples at room temperature for 5 minutes



Shake samples again 10-20 times



How do know if the panel is good enough?

- Look at the standard deviation (SD)
 - A SD between 1-3 is good
 - If it is over 3 look at SPC Cts, check for SPC Cts over 34.1
 - This could be an indication of cartridge variability and not necessarily cause by the sample (Blakemore 2011)
 - Repeat testing for samples with SPCs over 34.1 and replace results
- Look at the mean Ct for probe A
 - 23 or less is good and will allow for some inevitable DNA degradation with limited false negative results considering shipping conditions are not extreme and panels are tested upon arrival at testing sites



Inactivation Verification

- Completed inactivation verification form >>signed by lab manager
- Copies of 2 unloaded Negative MGIT reports (day 1-42 and day 43-84)



THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

GXPT/PP/011, Version 2.0, Effective date: 01-Apr-2022

18

UGANDA
Supranational
Reference Laboratory®

Timely Accurate Diagnostics for a TB-Free Africa

Inactivation Verification documents

BACTEC MGIT 960 Unloaded Negatives Report

Instrument Number	Current Date/Time	Temperature			Software Version	Page Number
		A	B	C		
2	04-06-2016 09:13	36.6°C	37.0°C	36.7°C	V3.06C	1

Tube Position	Accession Number	Sequence Number	Growth Unit	TIP	Removal Date	Protocol Length	Start of Protocol
A/S16	S531L-1607	430129547306	0	47;15	04-06-2016	42	02-18-2016 17:18
A/S17	7164-1606	430129547290	0	47;15	04-06-2016	42	02-18-2016 17:19
A/S18	S522L-1605	430129547274	0	47;15	04-06-2016	42	02-18-2016 17:19
A/S19	H526Y-1604	430129547313	0	47;15	04-06-2016	42	02-18-2016 17:19
A/S20	H526D-1603	430129547297	0	47;15	04-06-2016	42	02-18-2016 17:19
A/A01	35810-1602	430129547281	0	47;15	04-06-2016	42	02-18-2016 17:19
A/A02	35811-1601	430129547329	0	47;15	04-06-2016	42	02-18-2016 17:19

2016-A
Second 42days
day 43-84

BACTEC MGIT 960 Unloaded Positives Report

Instrument Number	Current Date/Time	Temperature			Software Version	Page Number
		A	B	C		
2 78	02-01-2016 14:06	36.6°C	36.9°C	36.6°C	V3.06C	1

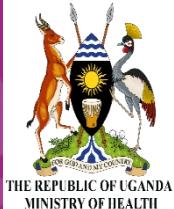
Tube Position	Accession Number	Sequence Number	Growth Unit	Tube Status	TTD	Date Positive	Protocol Length	Start of Protocol
A/R08	kans-1600	430129547321	10319	+	14;19	01-21-2016	42	01-06-2016 17:23

Labeling

- Print sheets of labels
- Design a panel/sample naming scheme
- ILB/CDC/NTRL/SRLs Scheme



- Label each sample in separate area to ensure samples are not mixed up.
- Label storage bags well and keep sealed tight before labeling
- After labeling store in bags of 100 tubes of same sample



Pack panels

- Take all labeled samples and create panels by adding one of each sample to a small zipper bag and sealing
- QC for panel packing
 - Take one bag of 100 for each of the 5 samples
 - Pour each one out into a container in numerical order
 - Count out 100 small zipper bags
 - Take one bag and place one of each sample in it, seal and place in container at the end
 - After a hundred bags are packed if all samples are packed and no odd one remain in any container rest assured each panel likely contains one of each sample
 - If odd tubes remain sift through the 100 panels and find the mis-packed panel and correct
 - Pour panels into consolidation bin for storage
 - Repeat starting with empty containers



Forms

Xpert® MTB/RIF Performance Evaluation Result Form

Performance Evaluation Panel ID: 2016-C Country: _____ Due Date: 27 January 2017

Instructions: Fill in the circles below corresponding to the results from testing each sample provided. Document the error code in the Error Code column for tests resulting in error. Record the cycle thresholds (Ct) in the cells provided for all successful tests.

Name of Site	Date Panel Received	Date Results Reported															
Site PT-ID Number																	
Site Contact Person	Date of Last GeneXpert Instrument Calibration or Installation																
Contact Email	Xpert MTB/RIF Cartridge or Pouch Lot No:																
	Xpert MTB/RIF Cartridge Expiry Date:																
Test Sample ID	Date Tested	TB Detection Result		Rif Result	Uninterpretable Result		Cycle Threshold (Ct) Value										
		D T E R O W N Y L O W	V Y R Y L O W	M E D I U M	H I G H	N A	D T E R O W N Y L O W	I N V I R U S T	N D E R O W N Y L O W	E R R O R	I X O X C O D E	Probe D	Probe C	Probe E	Probe B	SPC	Probe A
Example	5/15/2016	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	23.4	23.2	24.1	23.9	26.5	23.1
2016-C-1		<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						
2016-C-2		<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						
2016-C-3		<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						
2016-C-4		<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						
2016-C-5		<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						

Comments: _____

Submit this form electronically by 27 January 2017 to your PTCC's email

ILB-500-FXXA v00

Effective Date:



INSTRUCTIONS:

If testing cannot take place immediately, store Xpert® MTB/RIF performance evaluation test packet in the dark at 2-8°C until testing.

1. Inspect

Please use the following list to determine if all performance evaluation test packet components are present.

- 1 plastic bag containing 5 labeled sample tubes
- 5 individually wrapped transfer pipettes
- 1 report form

Inspect each of the 5 sample tubes to ensure a small button of dried blue material is present at the bottom of each tube. Please note the absence of the blue material from any of the sample tubes in the comments section of the results form.

2. Test

Caution: These samples contain nonviable strains of mycobacteria and should be treated as infectious material. These test samples should only be opened in a laboratory where the Xpert® MTB/RIF assay is performed, using the same precautions and safety measures employed when testing clinical samples.

Testing Instructions:

- Treat performance evaluation samples as clinical patient samples and test the DTS samples in the next 5 available GeneXpert modules.
- Only rehydrate the number of DTS samples that can be tested immediately, store remaining samples in the dark at 2-8°C until modules are available for testing.
- Rehydrate the dried tube specimen (DTS) samples.
 - Use the individually wrapped transfer pipettes included in the performance evaluation test packet to add 2.5 ml of sample reagent (SR) from the Xpert® MTB/RIF assay kit to each of the 5 sample tubes.
 - Avoid cross-contamination by using a separate transfer pipette for each sample, one bottle of sample reagent per sample and only having one sample open at a time.
 - Tighten the caps on the sample tubes.
 - Shake each sample vigorously 10-20 times.
 - Incubate the rehydrated samples for 10 minutes at room temperature.
 - After 10 minutes has elapsed shake each sample vigorously 10-20 times and incubate for an additional 5 minutes at room temperature.
 - Aspirate the rehydrated sample up to mark on pipet provided with Xpert® MTB/RIF assay kit (2 ml).
 - Add sample to large open port in the Xpert® MTB/RIF assay cartridge.
 - Close the lid and repeat sample addition for the remaining samples.
 - Test immediately.

3. Report

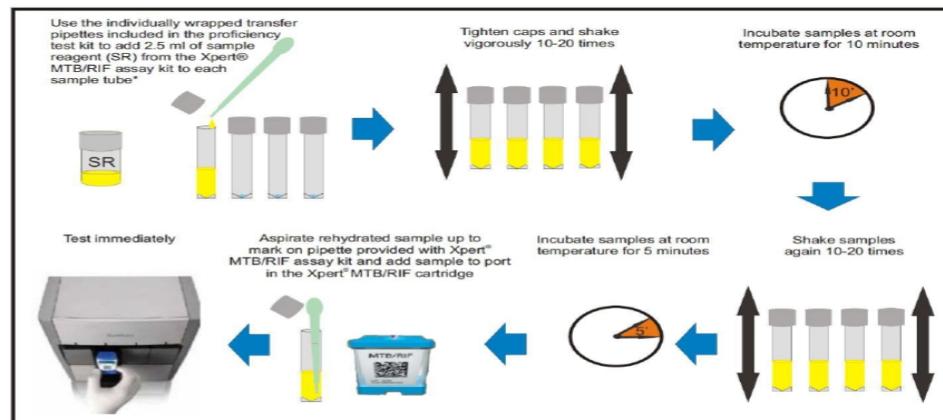
- Please complete the Xpert® MTB/RIF Performance Evaluation Result Form included in the test packet or respond to the electronic reporting email you received from your Xpert Performance Evaluation Country Coordinator.
- If completing the paper result form included in this packet, completely fill the circles indicating your results.
- If completing the electronic result form, use the drop down menu in each cell to select the answer corresponding to your test result.
- If a test results in an error, document the error code in the Error Code column.
- If a run fails or you are unable to completely test a sample please note the reason in the comments section on the report form.
- Submit the completed form to your country's Xpert performance evaluation coordinator at the email address on the bottom of the report form.
- A result summary will be returned to you via your Xpert Performance Evaluation Country Coordinator.

Forms

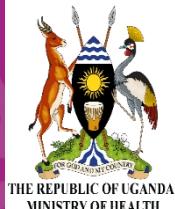
Testing Xpert® MTB/RIF Dried Tube Specimen (DTS) Performance Evaluation Samples

Caution: Xpert MTB/RIF DTS samples contain nonviable strains of mycobacteria and should be treated as infectious material. The DTS samples should only be opened in a laboratory where the Xpert® MTB/RIF assay is performed, using the same precautions and safety measures employed when testing clinical samples.

- Performance evaluation testing should be incorporated into the flow of clinical testing.
- Only rehydrate the number of samples that can be tested immediately.
- Time between sample rehydration and testing should not exceed 30 minutes.
- Prior to testing, store samples at 2-8°C in the dark.



*Avoid cross-contamination by using a separate transfer pipette for each sample, one bottle of sample reagent per sample and only having one sample open at a time.

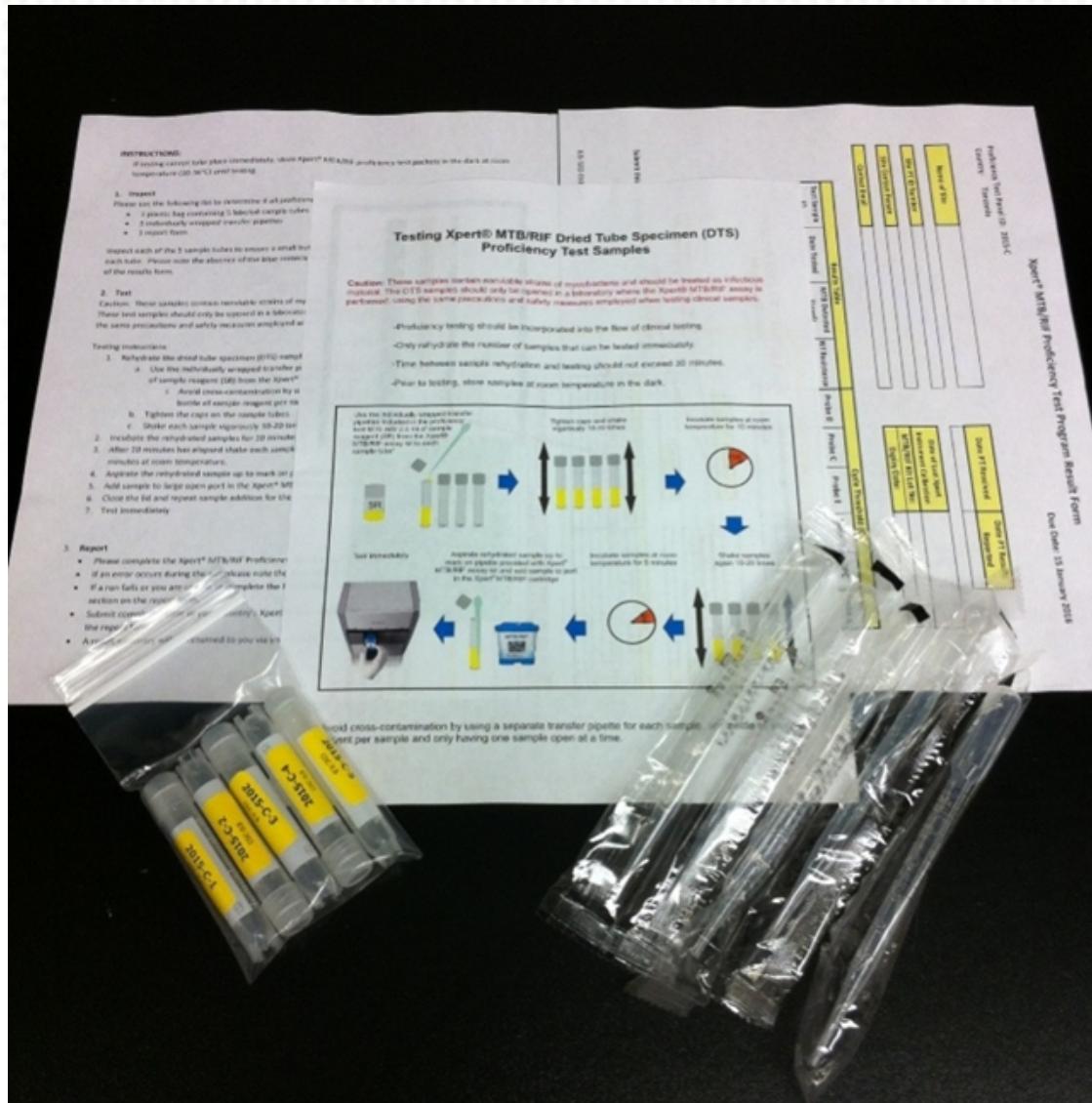


PT Packets

- Include
 - Instructions, job aids and forms
 - All reporting forms are specific to country or region
 - 1 panel
 - Five sterile 3 ml graduated transfer pipettes
- PT Packet QC
 - Pack packets for one country or region at a time
 - Count out number of panels needed for each country/region
 - Collate instructions job aid and report form slide into zipper bag, add panel and 5 pipettes>>seal and place in shipping box
 - If all panels are used when all country/region specific forms are used >> rest assured panels are correctly packed
 - If panels or forms remain when the other is depleted, sift through the PT packets and correct



PT Packets



Ship and send email

- Send shipping documents to PTRCs
- Email PTRC compilation template and digital result form
- Be available for questions
- Send reminder a few weeks before submission due date
- Confirm each received result with email
- Analyze results as described in DTS PT Panel Data

Analysis and Report Generation document.



Assessment

1. How do you know if the panel is good enough?
2. Why do you need to inoculate the inactivated sample for 2 cycles/ 84 days?



Summary

1. The SD and Ct values for the SPC and Probe A are useful indicators of the panels that are good enough
2. Inoculating the inactivated sample for 2 cycles/ 84 days is essential for verifying the inactivation process



References

- ISO 13528:2005, *Statistical methods for use in proficiency testing by interlaboratory comparisons*
- ISO 15189:2012, *Medical laboratories – Particular requirements for quality and competence*
- ISO Guide 34, *General requirements for the competence of reference material producers*
- ISO Guide 35, *Reference materials – General and statistical principles for certification*
- ISO/IEC 17043 First edition 2010-02-01
- Guide 34, ISO Guide 35 and ISO 13528 (homogeneity and stability)
- ISO/IEC Guide 98-3, *Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*



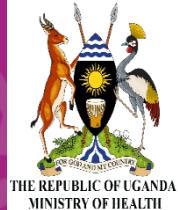
Acknowledgments



KOFIH
Korea Foundation for International Healthcare
한국국제보건의료재단



Timely Accurate Diagnostics for a TB-Free Africa



GXPT/PP/011, Version 2.0, Effective date: 01-Apr-2022

30



Timely Accurate Diagnostics for a TB-Free Africa