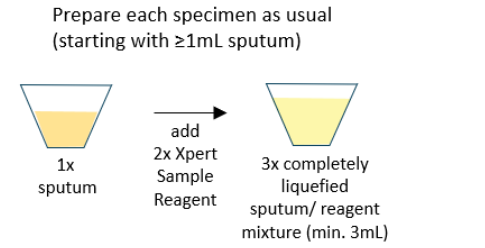
Job Aid- Pooled testing of sputum samples for TB detection by the Ultra assay

Scope: Pooled testing can help reduce the time and cartridges required for diagnostic testing using the Xpert MTB/RIF Ultra assay. It is recommended that pooled testing be used for individuals at lower risk for TB. Pool testing should typically be used in populations with a TB positivity rate of <15%.

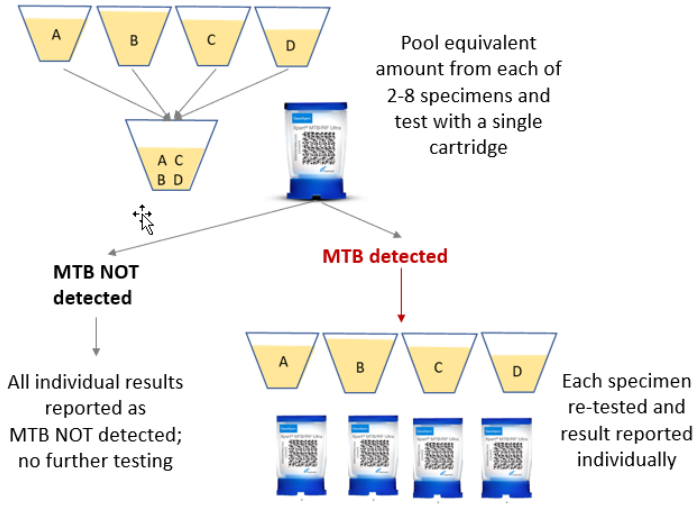
Note: It is recommended that testing be performed with pools of either 2, 3, 4 samples or as needed; If the positivity in the population is >10%, it may be preferable to use a pool size of 2; for positivity of <10%, typically a pool size of 3 to 8 is recommended based on the risk level of patients. Please contact the reference lab if you need guidance on how to choose the best pool size.

Schematic overview of pooled testing:



Pool size	Volume of each sample/ reagent mixture to add to pool	Volume of pooled mixture for testing	Volume of individual sample/reagent mixture remaining (for individual re-testing if needed)
2-4	1 mL	2-4 mL	≥2mL
>4	0.5 mL	>2.5 mL	≥2.5mL

Note: Person only submits one sputum specimen- typically no need for repeat collection



Type of samples to be used for pooled testing and criteria for pooling

- Only specimens sent for initial TB diagnosis;
- Sputum specimens only;
- Sputum specimens with a volume of 1.0 mL or more;

Type of specimens to be tested individually

- Extrapulmonary or non-sputum pulmonary specimens;
- Samples with a volume of less than 1.0 mL;
- Specimens from persons undergoing TB treatment or for rifampicin testing after an initial positive MTB or smear result should be tested individually;

Materials needed:

- Ultra cartridges (Xpert MTB/RIF Ultra cartridges, Cepheid).
- Xpert testing register (provided by reference lab).
- Pasteur pipettes (or repeat pipette with pipette tips); either provided with Ultra cartridges or provided separately.
- Xpert Sample Reagent (provided with cartridges); may be used for more than one specimen if aseptic procedures are respected to avoid contaminating the reagent container between specimens.

- Timer (to time the duration of Xpert sample reagent in sputum sample)
- Sputum mugs (to create pooled specimen/reagent mixture).
- Bold marker (for writing testing numbers on pooled sputum mug and Xpert cartridges)
- Xpert instrument.
- Waste disposal containers (for specimen preparation area and for Xpert testing area)
- Materials to disinfect working surfaces.

Procedure

IMPORTANT Note: Before starting testing, ensure your workspace is clean, free of clutter and ONLY specimens to be involved in the pooling are present in the working area. This is critical to avoid mistaking one sample for another and to avoid cross contamination.

1. Collect/receive samples following routine protocols.
2. At check-in, sort request forms for those eligible for pooling.
3. Visually assess collected samples to identify those with sputum volume ≥1.0mL.

Notes:

a. If sputum volume is less than approximately 1.0mL, exclude this sample from the pool and process individually (this is to ensure there is enough specimen/reagent mixture left for subsequent individual testing as needed, if the pool result is positive or invalid/error/no result).

b. **If the sample volume is ≥4mL, aliquot about 2mL into a new labeled sputum mug (See Bench Aid for Aliquoting sputum samples-BA-AG-8) and use for testing to maximize reagent.**

4. Create patient and specimen identification numbers and enter these into the Xpert register.

a. For pooled testing, the Patient ID field (in both the Xpert register and the Xpert instrument) is filled using the first name of each patient in the pool separated by a comma. e.g., for Mbacham Einstein, Ebot Rudinger, Tsala Mccay, Penn Mercy, this would be: **Patient ID field is: Mbacham,Ebot,Tsala,Penn**

Note: For pools of six (6) use the initials of the patient e.g. for the example above the patient ID field will be ME,ER,TM,PM… if it were a pool of six.

b. The Sample ID field is composed of the pooled ID (in both the Xpert instrument and Xpert register). This ID is created using "P(SN)" to indicate "pool serial number" followed by the last number of each specimen's record number, with a dash in between, e.g., for a 00107, 00129, 0117, 00189 group of specimens, this would be:

Sample ID: P001-7-9-7-9 (assuming this is pool number 1)

Note: Typically, the pool serial number should be assigned either from the beginning of when pooling started or at the beginning of the year and restart at the beginning of the next year. This pool serial number helps the laboratory to track the number of pools performed.

c. Enter the complete sample record number and initials of each patient's name i.e 00107-ME, 00129-ER, 00117-TM, 00189-PM into the Notes field in the Xpert machine

d. Enter each individual patient's name and sample ID tested as part of this pool in the next rows below the information for the pool test. The Xpert register for the above example should look like this:

SN	Registration no.	Patient ID	Age	Sex	Specimen ID (Sample ID)
1	-	Mbacham,Ebot,Tsala,Penn	-	-	P001-7-9-7-9
2	00107	Mbacham Einstein	29	M	1BRH-00107-ME
3	00129	Ebot Rudinger	46	M	1BRH-00129-ER
4	00117	Tsala Mccay	65	F	1BRH-00117-TM
5	00189	Penn Mercy	80	F	1BRH-00189-PM

5. Label a new sputum mug with the Pool ID, for example, P001-7-9-7-9. This container will be used to create the pooled sample/reagent mixture. Place this labeled container behind the specimens to be pooled.

6. Prepare each of the individual samples to be pooled according to the normal specimen preparation procedure for the Xpert MTB/RIF Ultra assay, i.e.

   i. For each specimen: using a new sterile Pasteur pipette (or pipette with tips), add 2 volumes of Xpert Sample Reagent to 1 volume of sample and then close the container. For example, if the sputum volume is 1.5 mL, add 3.0 mL for a total volume of 4.5 mL (note that during pooling, you will add 1.0 mL to the specimen pool, so that 3.5 mL will remain in case the pool Ultra result is positive and you need to re-test that sample individually for Ultra). (Note: If the sputum specimen volume is close to 1mL, add slightly more than 2mL Sample Reagent. This is to ensure you will have slightly more than 2mL of the sample/reagent mixture remaining in case you need to re-test individually).

Important Notes:

- Be cautious not to contaminate the remaining Xpert Sample Reagent and close it immediately after use. If the Sample Reagent is only pipetted with a sterile pipette and never contacts the specimen, specimen container or a contaminated pipette, it can be used as a source of Sample Reagent for other specimens. This will help to extend the availability of Sample Reagent for specimen pooling.
- If using a Pasteur pipette, use a new pipette for each specimen and keep the pipette in the pipette plastic between steps. Make sure you put back the used Pasteur pipette in the pipette plastic and keep it aside the sample as it will also be used to transfer the sample/reagent mixture to the pool container for that specimen.
- If using a standard pipette, discard the used pipette tip immediately after use.

   ii. Mix the specimen and Sample Reagent thoroughly by shaking vigorously 10 to 20 times. Incubate for 10 minutes at room temperature.

   iii. Mix thoroughly and incubate for an additional 5 minutes at room temperature.

   iv. Visually inspect the specimen/reagent mixture. If it is completely liquefied, proceed to the next step. If it is only partially liquefied, mix again thoroughly and let stand for an additional 5 minutes or until completely liquefied. Avoid pipetting solids or debris into the Ultra cartridge to avoid errors.

7. Once each of the individual sample mixtures are liquefied, create the pooled sample by adding each sample/reagent mixture to the labeled sputum mug dedicated to the pooled sample. Carefully open the individual sputum mug and depending on the pool size, transfer an amount from each sample as indicated on the table below into the dedicated pooled sputum mug using the same Pasteur pipette used in step 6(i) above. Close the lid of the individual sputum mug before opening the next sputum mug for the next sample.

Pool size	**Volume of each sample/reagent mixture to add to pool**	**Volume of pooled mixture for testing**	**Volume of individual sample/reagent mixture remaining (for individual re-testing if needed)**
2-4	1 mL	2-4 mL	≥2 mL
4-8	0.5 mL	>2.5 mL	≥2.5 mL

Note: The maximum pool size should be a pool of 8.

8. Repeat step 7 for each sample/reagent mixture to be included in the pool.

9. Set aside the sputum mug containing the individual samples/reagent mixtures for later use if necessary (if the pooled test result is positive or invalid, each sample must be retested individually).

10. Label the cartridge to be used for pool testing with the pool information from the sample ID field (e.g., P001-7-9-7-9).

11. Using one of the transfer Pasteur pipettes used for the individual samples, aspirate the liquefied sample pool just above the line on the pipette (just above 2 mL). If there is insufficient sample volume, do not proceed with testing. (Note: If the test is run with <=2mL, it may generate an error)

12. Perform an Xpert Ultra test using the pooled sample according to the Cepheid protocol. Verify that the cartridge is labeled with the same Pool ID as the one on the pooled sample container (For example, P001-7-9-7-9).

13. To enter specimen information to the Xpert machine,
    a) If DataToCare software is installed in your Xpert machine, follow these steps:
        I. In the Disease and method section, check to confirm the chosen field: Tuberculosis > molecular > Xpert MTB RIF Ultra.
        II. For the Patient field, type the first letter of the name then click 'New patient' and enter the information as follows; In the field for 'First name', enter <POOL> while in the field for 'Second name", enter the first names of each patient in the pool separated by a dot' e.g. <Mbacham.Ebot.Tsala.Penn>
        III. Enter female (F) for Gender of a pool. (Use this for all pool tests)
        IV. Enter Age as 99 and Save. (Use this for all pool tests)
        V. For Prescriber, enter or choose name as 'PoolTesting', with phone number 699999999.
        VI. Select Sample Type: click on the drop-down list and choose the type <SPUTUM>

VII. Input Specimen ID in the Sample ID field as above (point 4b): e.g. Sample ID: P001-7-9-7-9

The Data to Care fields should now look like this for the pooled test:

Data to Care field	**Entry for pooled test**
Patient – First Name	POOL
Patient – Last Name	From 4b (eg Mbacham.Ebot.Tsala.Penn)
Sex	Female
Age	99
Prescriber	PoolTesting (phone: 699999999)
Specimen ID	from 4b above (eg. P001-7-9-7-9)

VIII. Health facility: choose the health facility name from the drop-down list and double click on the name of the DTC, if not displayed; manually write the name of the health facility.

IX. Enter information on the <Test context> e.g. Diagnostic testing, the operator and the date sample was submitted.

X. Click on <Send Order> to proceed with testing.

XI. Complete the record of the patients by entering sample record number and initials of each patient's name e.g. 00107ME,00129ER,00117TM,00189PM in the Notes section of the Xpert instrument. This information is the same as entered into the Xpert Register, as described in section 4d.

Field	**Xpert instrument entry**
Notes	1BRH-00107-ME 1BRH-00129-ER 1BRH-00117-TM 1BRH-00189-PM

Note: If pool result is MTB DETECTED or INVALID or ERROR, all detailed information for each patient in the pool will be entered to the Data-To-Care during testing the individual samples.

b) If DataToCare software is not installed or out of use, proceed as follows:

XII. To enter specimen information into the GeneXpert instrument, use the following: For the "**Notes field**" in the GeneXpert instrument, using the Barcode scanner of the GeneXpert instrument, point it on the QR code of the first participant in the pool to scan the QR code. Repeat this for all the participant QR codes in the pool. Put a dash (-) and add the initials of the participants corresponding to the scanned QR code as shown below.

Field type	**Lab Register**	**Xpert instrument**
Patient ID	Names (e.g. Mbacham,Ebot,Tsala,Penn)	Names (e.g. Mbacham,Ebot,Tsala,Penn)
Sample ID	P001-7-9-7-9	P001-7-9-7-9
Notes	N/A (see 4d above)	1BRH-00107-ME 1BRH-00129-ER 1BRH-00117-TM 1BRH-00189-PM

14. When the results are ready, save them using the steps below:

a. For a positive pool (MTB Detected)

i. If the pooled sample is "MTB Detected," with any grade, record the result in the Xpert register with the pooled sample ID as MTB Detected and retest all three samples individually.

ii. Repeat the test for each patient in a positive pool individually, for each sample:

- Record the serial number in the Xpert register for each line where an Ultra cartridge is used.
- Label a new cartridge with the individual sample ID, e.g., 00107-ME, as on the individual sputum mug.
- Using a new Pasteur pipette, aspirate the liquefied individual specimen/reagent mixture reserved in step 7 just above 2 mL and transfer to a new Ultra cartridge. Label the cartridge with the patient registration number and initials.
- Enter the patient information into the Data to Care system as for a standard individual test or directly into the GeneXpert instrument (as appropriate), using the following:

Patient ID field	Patient name, e.g. Mbacham Einstein
Sample ID field	1BRH-00107-ME

iii. Once the individual test results are available, record the individual results in the Xpert register with the individual sample ID as obtained from the instrument. Example of an Xpert register for a positive pooled test result. Each time an Ultra cartridge is used, the serial number column is filled in. This allows the laboratory to track the use of the Ultra cartridges.

iv. After repeating the individual tests, complete the Xpert results log with the individual test results as shown below.

SN	Sample ID	Xpert results	If MTB (RR-IND, NOT)
1	P001-7-9-7-9	MTB Detected Trace	IND
2	1BRH-00107-ME	MTB Not Detected	N/A
3	1BRH-00129-ER	MTB Very Low	NOT
4	1BRH-00117-TM	MTB Not Detected	N/A
5	1BRH-00189-PM	MTB Not Detected	N/A

v. Prepare individual results to be given to the client when they come to pick up their results.

Note: All samples in a positive pool should be tested to identify the positive before results from that pool are given out.

b. For a pool with INVALID result or ERROR, follow the same steps as for a positive pool.

If the `pooled sample gives an error or invalid result, record this result in the Xpert register next to the pooled sample ID as "Error", or "Invalid", or "No Result", depending on the result given by the instrument and repeat the test individually following the same steps as for a positive pool. **Note:** You may decide to repeat a pool with an error or invalid result if you

consider that after performing a pool repeat you will still have enough samples to perform individual tests for each specimen in the case of a positive.

c. For a negative pool (MTB Not Detected) follow the steps below.

i. If the test result is "MTB Not Detected", all three samples are MTB not detected. Record the result in the logbook with the pooled sample ID and the individual sample IDs as MTB not detected. Example of a record for a negative pooled test result. Each time an Ultra cartridge is used, the serial number column is filled in. (This helps the laboratory keep track of Ultra cartridge usage)

SN	Sample ID	Xpert results	If MTB (RR-IND, NOT)
1	P001-7-9-7-9	NOT	N/A
	1BRH-00107-ME	NOT	N/A
	1BRH-00129-ER	NOT	N/A
	1BRH-00117-TM	NOT	N/A
	1BRH-00189-PM	NOT	N/A

ii. Prepare the individual results for the patient and give them to the patient when they come to pick up their results.

Notes:

- The purpose of individually retesting each specimen from a positive pool is to identify which specimen(s) is/are positive; If the individual specimen result identifies one or more positive specimens, report the individual specimen result obtained by following the normal procedure for reporting a positive result.
- Any positive result (MTB Detected- Trace, Very Low, Low, Medium, High) indicates that the patient should be started on TB treatment immediately according to national guidelines.
- While it is rare, there may be times when you test each sample from a positive pool individually and none of them have a result of "MTB Detected." If all individual samples have a result of "MTB NOT detected," collect a second sample from each patient and retest each individually. If the test result identifies the sample with "MTB Detected," report the result as usual. If retesting of all the specimens in the positive pool does not result in any "MTB detected" result, then report each result as "MTB NOT detected." Add each of these patients to the patient follow-up record and continue to follow each patient until they are better, including providing additional Ultra tests as needed.
- It is very important to test the individual specimens from any positive pool within 4 hours of adding the sample reagent. If this cannot be done, store the specimen/reagent mixture in the refrigerator and test within 24 hours. Failure to test within the stated time may lead to degradation of the DNA and could result in a false positive pool, for example.
- Contact the reference laboratory if you have any questions or require further information