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Xpert MTB/RIF Ultra testing from tongue swabs – Diluted SR method

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We use this protocol and it's working

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Abstract

This standard operating procedure (SOP) outlines the methodology for processing dry dorsum tongue swabs with Xpert MTB/RIF Ultra (Ultra) (Cepheid, Sunnyvale, CA). The procedure is intended as a consensus protocol for clinical evidence generation to evaluate whether tongue swabs can be used as an additional sample type to diagnose tuberculosis (TB) using Ultra. Tongue swabs will be collected for Ultra testing at the clinical facility. Please refer to the tongue swab collection SOP. Several groups have shown that the use of Xpert Sample Reagent (SR) at a starting concentration of 100% can result in decreased sensitivity for the detection of *Mycobacterium tuberculosis* from tongue swabs. This protocol makes use of diluted SR in phosphate buffer (PB), with a starting concentration of SR of 66%.

Attachments

PNG

Cepheid Dilute SR Pr...

548KB

Guidelines

Personnel testing the tongue swabs must wear a fitted N95 mask to protect from TB aerosols, eye protection (safety glasses or face shield), and disposable gloves. These should be worn during the entire collection process and should only be removed after leaving the ward/ location where the sample was collected. Wash hands thoroughly after handling samples.

Materials

1. Disposable gloves and appropriate personal protective equipment
2. Tube rack
3. Refrigerator or insulated cool box with ice packs at 2-8°C
4. Vortex mixer
5. 1000 µl pipette
6. 1000 µl sterile filter pipette tips
7. Serological pipette and consumables
8. Sterile conical tube: 15 mL or 50 mL, or other size that can fit 12 mL of volume.
9. Xpert MTB/RIF Ultra cartridges
10. GeneXpert instrument (GeneXpert II or GeneXpert IV or GeneXpert XVI)
11. Phosphate buffer (PB), pH 6.8

Before start

Upon receipt at the laboratory, keep the collected tongue swab at 2-8°C until ready for processing. **Processing and testing should be done within 24 hours of collection.** If processing cannot be done within 24 hours, please store the swab at -80°C for later testing.

For stored swabs that will be tested from frozen, please remove the swabs from the freezer and keep on ice (2-8°C) for at least ten minutes and until ready for testing.

Please refer to the attached schematic workflow.

Preparation of diluted 66% SR:PB

15s

1 Dilute SR in a 2:1 ratio with PB, daily in a biosafety cabinet.
Please use step 2 for testing of smaller batches of samples, and step 3 for testing of larger batches (up to 16 samples) or standardized daily preparation of diluted SR buffer

> **Note**
>
> Stability of the SR:PBS buffer has not been established. Please prepare this buffer fresh daily. After the tongue swabs have been tested, store the remaining SR:PB in a refrigerator until the end of the day. Discard the diluted SR:PB buffer at the end of the day.

2 **Method A: For testing of smaller batches of samples**

2.1 Label a sterile conical tube with the date of the diluted SR:PB preparation.

2.2 Depending on the number of samples to be tested, add the volume of SR and PB to the labeled conical tube as per the table below. The total volume allows for 10% overage.

A	B	C
Number of samples to be tested	Volume of SR (mL)	Volume of PB (mL)
1-3	2	1
4-7	4	2
8-11	6	3
12-16	8	4
17-20	10	5
21-25	12	6

2.3 Vortex (maximum setting) to mix for 5 to 10 seconds.

2.4 Use the diluted SR:PB buffer to test tongue swabs collected on the same day the buffer is prepared.

3 **Method B: For testing of larger batches of samples (up to 16 samples) or standardized daily preparation of diluted SR buffer**

3.1 Label a sterile conical tube with the date of the diluted SR:PB preparation.

3.2 Add the entire contents of Cepheid SR buffer (8 mL) from 1 bottle to the labeled conical tube.

3.3 Add 4 mL of sterile PB to the same conical tube.

3.4 Vortex (maximum setting) to mix for 5 to 10 seconds.

3.5 Use the diluted SR:PB buffer to test tongue swabs collected on the same day the buffer is prepared.

Xpert MTB/RIF Ultra Processing

4 As per local safety requirements for sputum processing, add 700 µl of the prepared diluted SR:PB buffer to each swab in the tube and close the tube lid.

5 Vortex to mix for 5 to 10 seconds.

6 Incubate the swabs at room temperature for 15 minutes. Vortex again halfway through the incubation time for 5 to 10 seconds (i.e. at between 7-8 minutes of the incubation time).

> **Note**
>
> The range of room temperature can be the same as for processing and testing of sputum with Xpert MTB/RIF Ultra.

7 Label the Xpert MTB/RIF Ultra cartridge with the appropriate sample identifier.

8 Prefill the cartridge with 1.5 mL of PB (undiluted).

9 Before loading the samples to the cartridge, briefly pulse vortex each sample. Over vortexing may generate bubbles that can adversely affect the test and cause difficulty for accurate pipetting.

10 Carefully add 0.5 mL of the SR:PB-treated swab to the cartridge, dispensing slowly against the interior wall of the cartridge to not generate bubbles.

11 Proceed with the instructions for “preparing the Cartridge” and testing that are found in the Xpert MTB/RIF Ultra manufacturer’s package insert.