STANDARD M10 MTB/NTM

STANDARD™ M10 MTB/NTM

REF M10-MTNT-01

INSTRUCTIONS FOR USE

For use with STANDARD™ M10 system

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SD BIOSENSOR

Contents

1. Intended Use

STANDARD M10 MTB/NTM is a multiplex real-time PCR test intended for use with STANDARD M10 system for the qualitative detection of *M. tuberculosis* complex (MTBC), and non-tuberculous mycobacteria (NTM) nucleic acids in human normal sputum or sputum sediment sample. Positive results are indicative of the presence of MTBC, and/or NTM; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Negative results should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. STANDARD M10 MTB/NTM is intended to be performed by trained users in both laboratory and near- patient testing setting.

2. Summary and Explanation

Tuberculosis (TB) is an infectious disease, which is caused by infection with *M. tuberculosis* complex organisms. It spreads to new hosts through the air from patients who have respiratory tuberculosis disease. Individuals newly infected would get symptoms from TB within weeks to months.

M. tuberculosis can cause disease in almost any tissue or organ in human body, such as lungs, kidneys, nerves, and bones. Among them, pulmonary tuberculosis, the lung tissue infection, accounts for the most. TB has different symptoms depending on the organ infected. For example, kidney tuberculosis causes symptoms of cystitis such as hematuria, difficulty urinating, and frequent urination. Spinal tuberculosis causes pain in the lower back. Tuberculous meningitis may cause symptoms such as headache and vomiting. In the case of the most common TB, pulmonary tuberculosis, symptoms such as coughing, chest pain, unintentional weight loss and fever.

NTM refers to mycobacteria except MTBCs and *M. leprae*. NTM may cause lung disease, lymphadenitis, skin-soft tissue-oste infection, and disseminated diseases. Unlike *M. tuberculosis*, NTM, the environmental saprophytic bacteria are free living, ubiquitously present in surroundings such as soil or water. For this reason, it was thought to be harmless environmental saprophytic and only dangerous to individuals with defective lung structure or the immunosuppressed, but recently, following facts increase concern for NTM infection; number of NTM disease incidence over last two decades has been risen, long term treatment with multiple antibiotics increases antibiotic resistance, and recurrence rate is high that leads to persistent chronic infection.

In general, drug-sensitive TB can be effectively treated with a standard multi-drug administration containing first-line antibiotics and second-line antibiotics. In contrast, the NTM bacteria have significant heterogeneity in their susceptibility to standard anti-tuberculosis drugs. Therefore, the treatment for NTM diseases should be differ from the treatment for TB, and this is the reason why identification of MTBC and NTM is required.

[Cartridge Description]

STANDARD M10 MTB/NTM is a molecular *in vitro* diagnostic assay that aids in the simultaneous detection and differentiation of MTBC and NTM DNA based on nucleic acid amplification technology, real-time PCR. STANDARD M10 MTB/NTM contains bacterial DNA extraction buffers and PCR reagents for the *in vitro* qualitative detection of MTBC and NTM bacterial DNA in human normal sputum or sputum sediment sample.

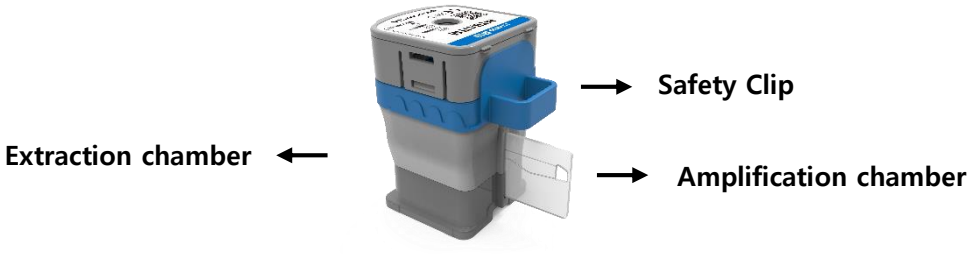

Figure 1. Layout of STANDARD M10 MTB/NTM cartridge

3. Principle of the Procedure

STANDARD M10 MTB/NTM is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from MTBC and NTM. STANDARD M10 MTB/NTM is able to perform on STANDARD M10 system. STANDARD M10 system automates and integrates sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in various specimens using molecular diagnostic assays. The system consists of STANDARD M10 Module and the STANDARD M10 Console with preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the nucleic extraction and PCR reagents and host the nucleic extraction and PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the STANDARD M10 user manual.

STANDARD M10 MTB/NTM includes reagents for the detection of DNA from IS6110 gene of MTBC strains and *ITS* gene of NTM in normal sputum or concentrated sputum sediment specimens. The cartridge contains an internal control for adequate processing of the sample and PCR reaction. The table below indicates which target is designed to be detected by which channel.

Table 1. Fluorescent channel of each target gene

Pathogen	Target gene	Channel
MTBC	IS6110	FAM
NTM	*ITS*	HEX
Internal control	Internal control (IC)	Cy5

4. Materials Provided

STANDARD M10 MTB/NTM contains sufficient reagents to process 10 specimens or quality control samples.

Table 2. Contents of STANDARD M10 MTB/NTM kit

	Contents	Quantity	Usage in each reaction
1	Cartridge	10	1ea
2	Quick Reference Instructions	1	-

5. Storage and Handling

Store STANDARD M10 MTB/NTM at 2 ~ 28℃ (36 ~ 82℉). If the cartridge has been refrigerated, it is recommended to leave the cartridge for 4 hours at room temperature (20 ~ 28℃, 68 ~ 82℉). Do not remove the safety clip of the cartridge and do not press the cartridge until actual use. Do not use a cartridge that has leaked or is wet. This kit should be stored at appropriate temperature and kept away from UV/sunlight. Under these conditions, cartridges can be stored until the expiration date, which printed on the label.

6. Materials Required but Not Provided

- STANDARD M10 with user manual
  At least one STANDARD M10 Console (Cat. No. 11M1011) and one STANDARD M10 Module (Cat. No. 11M1012)
- Sample collection and transfer tools
  - Sample container
  - Screw-capped tube
  - 0.067M Phosphate/H_2O buffer
  - Micropipette with filter tips
  - Pretreatment solution (PS) - STANDARD M10 Sputum Pretreatment Kit (Cat. No. 11PRT10A)
  - Disposable dropper - STANDARD M10 Sputum Pretreatment Kit (Cat. No. 11PRT10A)
  - Pretreatment tool - STANDARD M10 Sputum Pretreatment Kit (Cat. No. 11PRT10A)
- PPE (Personal Protective Equipment)
- Biohazard container

7. Warnings and Precautions

1) This kit is only for *in vitro* diagnostic (IVD) use.
2) For professionals use only.
3) Please read the Instructions for Use carefully before testing.
4) Improper specimen collection, transfer, storage, and processing may cause erroneous test results.
5) Do not remove the safety clip of the cartridge before use.
6) Do not press the cartridge until actual use.
7) Do not use a cartridge that has leaked or is wet.
8) Keep the cartridge away from UV/sunlight and keep dry.
9) Do not use the kit after its expiration date.
10) Do not shake, tilt, or invert the cartridge especially after pressing the cartridge to punch the seal. It may yield non-determinate results.
11) Do not use a cartridge with a damaged barcode label.
12) Do not reuse processed cartridges.
13) All patient samples should be handled as if these samples are infectious.
14) All materials should be considered potentially infectious and should be handled with precautions.
15) As this test involves extraction of bacterial DNA and PCR amplification, care should be taken to avoid contamination. Regular monitoring of laboratory contamination is recommended.
16) When using this kit, it should be operated strictly in accordance with the instructions and follow the technical requirements of the clinical gene amplification laboratory.
17) Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents.
18) When using a positive control and negative control, perform the test in a separated laboratory space to prevent contamination.
19) *M. tuberculosis* complex and non-tuberculous mycobacteria (NTM) cannot be diagnosed based solely on the results of this product,

and the confirmed diagnosis must be made by a specialist considering further tests or clinical results.

8. Specimen Collection, Transport, and Storage

Proper sample collection, transportation, and storage are critical to the performance of the test. Improper sample collection, inappropriate sample handling and/or transportation can lead to false results.

8.1. Specimen collection

Collect normal sputum following your institution′s standard protocol for sample collection and test normal sputum or concentrated/decontaminated sputum sediment.

Specimen Type :

- Normal sputum treated with Pretreatment solution(PS)
- Sputum sediment treated with Pretreatment solution(PS)

See Table 3 to determine adequate specimen volume when pretreating the two kinds of samples with Pretreatment solution(PS).

Table 3. Requirement of Specimen Volume

	Specimen Type	
	Normal sputum	**Sputum sediment**
Minimum Sample Volume	0.5 mL	
Specimen to Pretreatment Solution(PS) Ratio	1:2[1)]	

Note: [1)] Basically, specimen to pretreatment solution(PS) ratio is used in a ratio of 1:2, but in case of sputum sample with high viscosity, it can be used up to a ratio of 1:3.

8.2. Specimen Storage and Transport

Store and transport the specimen refrigerated temperature(2 ~ 8℃, 36 ~46°F), protected from light.

9. Procedure

9.1. Specimen procedure

9.1.1. Procedure for normal sputum

1) Prepare the normal sputum in sputum collection container and pretreatment solution(PS).
2) Open carefully the lid of the normal sputum collection container and remove solid particles that may affect the test.
3) Add approximately 2 times the volume of the PS into the normal sputum(1:2=normal sputum:PS) in the container and secure the lid. If the viscosity is still high, and not liquidized enough for testing despite PS application, add more PS up to a ratio of 1:3. For example, if normal sputum and PS are used at 0.5 mL and 1.0 mL, respectively, add 0.5 mL of PS to make the final 1:3 ratio.
   Note: More than 0.5 mL of normal sputum is required.
4) Vortex vigorously twice for 10 seconds to make sure normal sputum and PS are mixed completely.
5) Incubate the sample for 15 minutes at room temperature.

9.1.2. Procedure for concentrated/decontaminated sputum sediment

1) Prepare sputum sediment in sputum collection container and 0.067M Phosphate/H_2O buffer.
2) Carefully open the lid of sputum sediment collection container and add 0.067M Phosphate/H_2O buffer for suspension.
   Note: More than 0.5 mL of suspended sputum sediment is required for the further steps.
3) Vortex sufficiently until completely suspended, and transfer 0.5 mL suspended sediment to new screw-capped tube using micropipette.
4) Add approximately 2 times the volume of the PS into the suspended sputum (1:2=suspended sputum:PS) in the tube and secure the lid. If the viscosity is still high, not enough for testing despite PS application, add more PS up to a ratio of 1:3.
   For example, if suspended sputum sediment and PS are used at 0.5 mL and 1.0 mL, respectively, add 0.5 mL of PS to make the final 1:3 ratio.
5) Vortex vigorously twice for 10 seconds each and mix suspended sediment with PS.
6) Incubate the sample for 15 minutes at room temperature.

9.2. Starting the STANDARD M10 system

Note	For the detailed instructions, refer to the STANDARD M10 system User Manual. If you have scanned the cartridge barcode in the STANDARD M10 and the software version is not compatible, a 'Not Supported Device' error message appears. Update the software before proceeding the test**.**

1) Turn on the STANDARD M10 system.
2) Check the STANDARD M10 Console and the STANDARD M10 Module are connected and functional.

Figure 2. Power connection

3) Enter the User ID and Password on the Log In screen of the STANDARD M10 Console and click the Log In button.
4) Touch the STANDARD M10 Module to run on the Home screen.
(The door of the selected STANDARD M10 Module will automatically open for cartridge loading.)

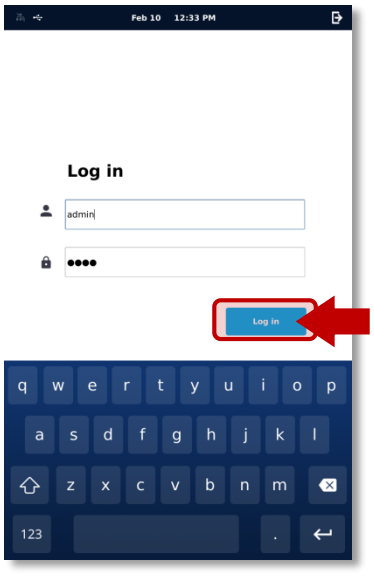

Figure 3. Log In screen

Figure 4. Home screen

5) Enter a Patient ID and Sample ID by scanning the barcode or using virtual keyboard on the M10 Console screen.
(Patient ID is optional. You can turn off the Patient ID option from the ‘Settings’.)
6) Enter a Sample ID by scanning the barcode of the specimen or using virtual keyboard on the M10 Console screen.
Make sure that the specimen tube cap is firmly closed when scan the ID barcode printed on the specimen tube.
(For quality control test, tick the QC check box.)
7) Scan STANDARD M10 MTB/NTM cartridge to be used. The STANDARD M10 Module automatically recognizes the assay to be run based on the cartridge barcode.

Figure 5. Entering Sample ID

Figure 6. Scanning a cartridge

9.3. Loading a sample into STANDARD M10 MTB/NTM cartridge

Caution	If the cartridge has been refrigerated, it is recommended to leave the cartridge for 4 hours at room temperature (20~28℃, 68~82℉). Start the test within 10 minutes of loading the sample into STANDARD M10 MTB/NTM cartridge.
Note	False negative results may occur if insufficient sample is added into the cartridge.

1) Remove the safety clip located underneath the lid of the cartridge.
2) Pierce the sealed cartridge by pressing down the lid until fully engaged into the cartridge groove.
3) Open the lid and check that the seal is completely punctured before loading a sample.
4) Carefully open the cap of the specimen tube or external control.
5) Refer to Figure 7. Transfer appropriate volume of the prepared sample into the barrel of pretreatment tool using disposable dropper with volume indication. Insert and press down the plunger of pretreatment tool, then it will inject 1 mL of the filtered sample into the cartridge.
6) After a few seconds, Sample Guide screen will automatically change to the Insert Cartridge screen. Touch the Sample Guide screen if you want to skip the guide.
7) Close the lid.

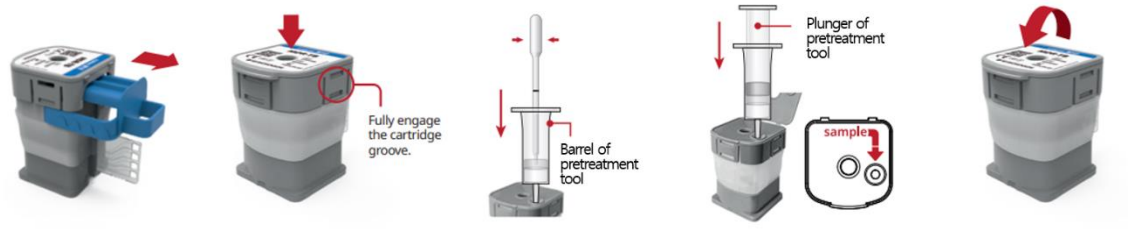

Figure 7. Loading a sample

Figure 8. Sample Guide screen

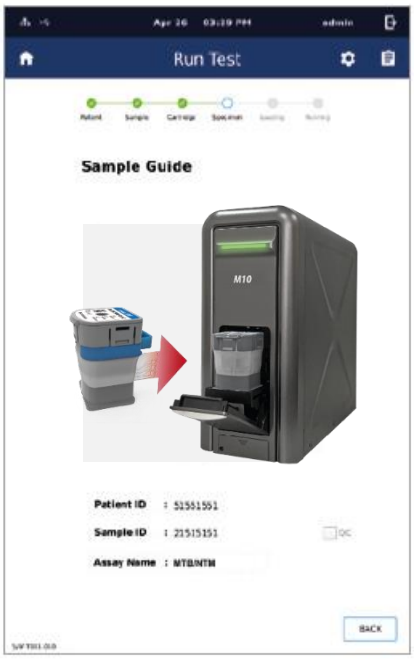

Figure 9. Insert Cartridge screen

9.4. Running a test

1) Load the cartridge on the selected STANDARD M10 Module with the Amplification chamber facing the inside of the module. (The status indicator of the selected module will blink green.)
2) Close the door completely.
3) After confirm the sample and cartridge information, touch the OK button on the screen. (Touch the Reset button to re-input the information.)
4) Assay starts automatically, and remaining time will appear on the screen.

Figure 10. Confirm the test screen

Figure 11. Running screen

5) When the run is finished, it switches to the Review screen and the result is displayed.

6) Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

7) To run another test, touch the Home icon and repeat the process. (If another STANDARD M10 Module connected to STANDARD M10 Console is available, you can start a new test while another test is running.)

10. Interpretation of Results

The results are interpreted automatically by STANDARD M10 Console and are clearly shown in the 'Review' screen. STANDARD M10 MTB/NTM provides test results based on the detection of respective gene targets according to the algorithms shown in Table 4.

Table 4. Interpretation of results

Result	MTBC (FAM)	NTM (HEX)	IC (Cy5)	NOTE
MTBC Positive	+	-	+	-
	+	+	+	**NTM Ct > MTBC Ct**
NTM Positive	-	+	+	-
MTBC / NTM Positive (Co-infection)	+	+	+	**NTM Ct ≤ MTBC Ct**
MTBC / NTM Negative	-	-	+	-
Invalid	+/-	+/-	-	-
Error	**No result**			-

Result (Summary screen)	Description
	**Target positive**
	Target negative
	**IC Valid**
	IC Invalid
	**Error**

Outcome (Home screen)	Result (Review screen)	Description
MTBC Positive		**MTBC positive**
NTM Positive		**NTM positive**
Co-infection		**MTBC and NTM positive**
Negative		**MTBC and NTM negative**
Invalid		**Invalid**
Error		**Error**

Result	Interpretation
MTBC positive	The MTBC target DNA is detected. • The MTBC signal has a Ct value within the valid range. • The NTM signal can be detected in MTBC target DNA, but it has a higher Ct value than MTBC signal (NTM Ct > MTBC Ct). • IC: Valid; IC signal is detected. - IC signal has a Ct value within the valid range.
NTM Positive	The NTM target DNA is detected. • The NTM signal has a Ct value within the valid range. • IC: Valid; IC signal is detected. - IC signal has a Ct value within the valid range.
MTBC / NTM Positive (Co-infection)	The MTBC and NTM target DNAs are detected. • The MTBC and NTM signals have a Ct value within the valid range. • The NTM signal has a lower Ct value than MTBC signal (NTM Ct ≤ MTBC Ct). • IC: Valid; IC signal is detected. - IC signal has a Ct value within the valid range.
MTBC / NTM Negative	The MTBC and NTM target DNAs are not detected. • IC: Valid; IC signal is detected. - IC signal has a Ct value within the valid range.
Invalid	The presence or absence of target DNA cannot be determined. Repeat test. • IC: Invalid; IC does not have a Ct value within valid range.
Error	The presence or absence of target DNA cannot be determined. Repeat test. • Target: no result • IC: No result

Note	- The NTM signal can be detected in MTBC target DNA. - If co-infection of MTBC and NTM is suspected, additional tests such as sequencing are recommended.

11. Quality Control

Quality Control procedures are intended to monitor cartridge and assay performance. If the controls are not valid, the patient results cannot be interpreted.

Internal control(IC): Ensures a proper sample has been applied, reagents in the cartridge are well functioning, there were no other interfering factors in the sample, and the procedure was performed correctly. If the IC fails where no MTBC or NTM is detected, the result is invalid.

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.
For external controls, it ts recommended to use the list below. Please comply with the information stated on the user manual.

-Positive control(PC): AMPLIRUN® TOTAL MTB CONTROL (SPUTUM)(Vircell, MBTC013)

-Negative control(NC): 1xPhosphate Buffer Saline (PBS)(No manufacturer restriction)

Products other than the mentioned substance can be used after being evaluated and validated for efficacy by each country or hospital independently.

12. Performance

12.1 Limit of Detection Test

The limit of detection test was assessed with two(2) MTBC strains (*Mycrobacterium tuberculosis* (H37Rv), *Mycrobacterium bovis*) and five(5) NTM strains (*M.avium, M.abscessus, M.intracellulare, M.masilience* and *M.kansasii*).
For the LoD test, seven(7) types of positive reference materials were serially diluted 2-fold using negative sputum sediment. Each of the seven(7) types of positive reference materials diluted into five(5) concentration ranges was repeated twenty(20) times over three(3) days. And the test was performed using two(2) different lots of MTB/NTM cartridge.
The verified LoD values for the tested bacteria were summarized in the Table below.

Table 5. Summary of the LoD test results

Specimen Type	Sputum sediment		
Limit of Detection Test	**Pathogen**	**Target**	**LoD (CFU/mL)**
	MTB	*M.tuberculosis*	0.255
		M.bovis	44.4
	NTM	*M.avium*	25.2
		M.abscessus	720
		M.intracellulare	80.7
		M.masilience	0.195
		M.kansasii	1,833

13. Limitations

1) The performance of the cartridge has been validated for normal sputum and sputum sediment specimens that has been liquefied, decontaminated and concentrated using NALC-NaOH. The use of other sample types may lead to false positive, false negative and/or invalid results.
2) A false negative result may occur if :
   - Sample concentrations is near or below the limit of detection of the test.
   - A specimen is improperly collected, transported or handled.
   - Inadequate numbers of organisms are present in the specimen.
   - Cartridges are exposed to improper environmental factors (temperature / humidity).
3) False positive results may happen from cross-contamination between patient samples, specimen mix-up and/or DNA contamination during product handling.
4) Qualitative detection of positive results in this kit does not indicate the presence of target gene. It is recommended to use other methods for confirmation at the same time.
5) This kit only classifies and identifies the MTBC and NTM. The test results are for clinical reference only. The clinical diagnosis and treatment of patients should be combined with their symptoms / signs, medical history, other laboratory tests and treatment responses considering.
6) Potential mutations within the target regions covered by the primer and/or probes of the test may result in failure to detect the presence of the pathogen.
7) This cartridge does not provide definitive confirmation of the results, as it may different from those detected by this device and associated with lack of clinical response to treatment. Specimens for which such clinical suspicion exists should be considered for further testing.
8) Exceeding volume limitations and/or deviating from procedural steps outlined in the "9.1 Specimen Procedure" section may lead to false positive, false negative and/or invalid results.

14. References

1) Centers for Disease Control and Prevention. (2009). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. Centers for Disease Control and Prevention.
2) Kent, P. T., & Kubica, G. P. (1985). Public health Mycobacteriology a guide for the Level Iii Laboratory. U.S. Dep. of Health and Human Services.
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4) Ratnatunga, C. N., Lutzky, V. P., Kupz, A., Doolan, D. L., Reid, D. W., Field, M., Bell, S. C., Thomson, R. M., & Miles, J. J. (2020). The rise of non-tuberculosis mycobacterial lung disease. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.00303
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6) Medecins Sans Frontieres(MSF) medical guidelines. (2022) Tuberculosis
7) World Health Organization. (2021). Tuberculosis (TB). World Health Organization. https://www.who.int/news-room/fact-sheets/detail/tuberculosis
8) CLSI EP17 A2; E valuation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline Second Edition

15. Symbols

REF	Reference number	LOT	Batch code
IVD	*In vitro* diagnostic medical device	CE	CE marking - European Conformity
	Consult Instructions for Use		Manufacturer
	Contains Sufficient for <n> Tests		Date of manufacture
	Caution	EC REP	Authorized representative in the European Community
	Note		keep dry
	Do not re-use.		Keep away from sunlight
	Temperature limit		Do not use if packaging is damaged
	Use-by date		

For further information on

STANDARD M10
MTB/NTM

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CE IVD

For *In Vitro* Diagnostic Use Only

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