# *Extraction of Mycobacterium tuberculosis* DNA from pretreated sputum using MagDEA Dx SV

(Example of using "MagDEA Dx SV," a prepacked reagent set for fully-automated nucleic acid extraction)

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## 〈Introduction〉

The cause of tuberculosis is a bacterium called *"Mycobacterium tuberculosis (M. tuberculosis)."* Tuberculosis is a life-threatening disease, starts with inflammation; as the inflammation progresses, then destroys the lung tissue thereby leading to dyspnea, and successively damages other organs. *M. tuberculosis* primarily infects the lungs, and is transmitted to other people by the airborne route to spread the infection.

Large amounts of bacteria exist in the sputum expectorated from patients infected with *M. tuberculosis* (patients with pulmonary tuberculosis). Therefore, testing of the sputum for *M. tuberculosis* is performed to determine whether a patient has tuberculosis. Generally, *M. tuberculosis* testing is conducted using a smear test in which a sputum sample is smeared onto a glass plate, stained, and examined by microscopy. However, when the bacterial quantity is small, microscopic examination may not always detect the bacteria. If the bacteria are not detected, the test is performed after cultivating the sputum. However, because the *M. tuberculosis* organism grows slowly, it takes 4 to 8 weeks until the cultivation result is obtained. In order to expedite detection of a minute amount of *M. tuberculosis,* detection methods using gene multiplication, such as the PCR method, have been employed in recent years.

We herein present the results of *M. tuberculosis* DNA extraction from a pretreated sputum sample using "MagDEA Dx SV," a reagent set for the novel fully-automated nucleic acid extraction system developed by Precision System Science Co., Ltd. (PSS).

## 〈Methods〉

Sputum samples were pretreated using either of the following methods, A or B:

A. *N*-acetyl-L-cysteine (NALC) treatment

1. To a sputum sample (3 mL) an equal volume of NALC-NaOH solution (2.5 g of *N*-acetyl-L-cysteine, 25 mL of 40%-NaOH, 25 mL of 1M sodium citrate, and 500 mL DNase/RNase–free water) were added, and the mixture was stirred at room temperature for 15 minutes.
2. Phosphate-buffered saline (PBS, pH7.4) (8 mL) was added to the above mixture, and the entire solution was stirred.
3. The above mixture was centrifuged at 3,200 g for 15 minutes, and the supernatant was discarded.
4. The pellets were suspended in PBS (pH 7.4) (900 µL).
5. The mixture was heated at 95°C for 20 minutes.

B. Proteinase K (PK) treatment

1. To a sputum sample (100 µL) an equal volume of proteinase K buffer was added, and the mixture was stirred for 10 seconds.
2. The resultant mixture was heated at 55°C for 1 hour.

Nucleic acids were extracted from the pretreated sputum suspension (200 µL) using the fully-automated gene test system geneLEAD XII Plus (PSS), the nucleic acid extraction reagent set MagDEA Dx SV and a nucleic acid extraction device (Company X). The extracted nucleic acids by both methods were tested for M. tuberculosis detection by real-time polymerase chain reaction (PCR).

## 〈Results〉

*Mycobacterium* (MTB) was spiked to 14 samples of NALC-treated (pretreatment method A above) *M. tuberculosis*-negative sputum suspensions (A to N), and extraction performances were compared by real-time PCR assay. Comparative data of Ct values obtained from the real-time PCR data are shown in **Fig. 1**. MagDEA Dx SV was capable of detecting MTB from all of the samples, and its capability was equal to or better than that of the nucleic acid extraction device from Company X. An extraction internal control (EIC) was also detected in all of the samples.

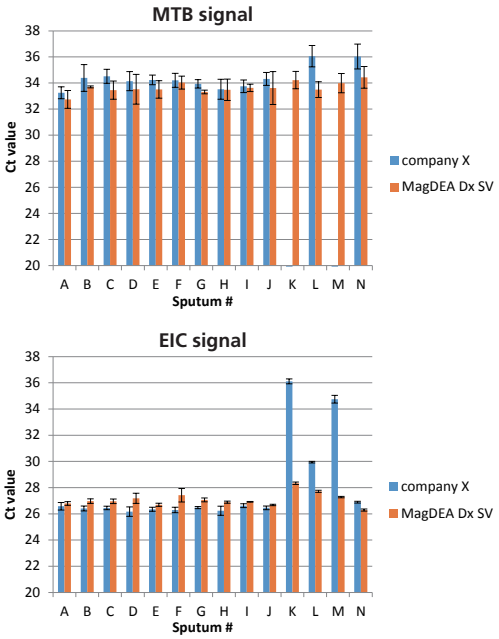


**Fig. 1 : Extraction testing using MTB-added sputum suspensions**

MTB was spiked to the PK-treated (pretreatment method B above) *M. tuberculosis*-negative, pretreated sputum samples (A to D) to achieve final MTB concentrations of 0, $10^2$, $10^3$, and $10^4$ copies/mL, and nucleic acids were extracted. Comparative data of Ct values obtained from the real-time PCR data are shown in **Fig. 2**. The minimal concentration detectable by MagDEA Dx SV was $10^3$ copies/mL; this detection sensitivity was the same as that of the nucleic acid extraction device of company X. The EIC was detected in all of the MTB concentrations examined.

**MTB**

| | Extraction reagent | Run | Sputum A | Sputum B | Sputum C | Sputum D |
|---|---|---|---|---|---|---|
| $10^4$ copies/mL | MagDEA Dx SV | 1st | 32.62 | 32.37 | 31.69 | 32.43 |
| | | 2nd | 32.12 | 31.96 | 33.67 | 31.67 |
| | company X | 1st | 32.13 | 31.70 | 31.31 | 30.96 |
| | | 2nd | 32.81 | 31.09 | 31.67 | 31.55 |
| $10^3$ copies/mL | MagDEA Dx SV | 1st | 35.25 | 35.15 | 35.27 | 36.34 |
| | | 2nd | 36.48 | 36.67 | 36.63 | 35.88 |
| | company X | 1st | 35.60 | 35.15 | 34.96 | 34.87 |
| | | 2nd | 36.34 | 35.89 | 33.71 | 35.64 |
| $10^2$ copies/mL | MagDEA Dx SV | 1st | nd | nd | nd | nd |
| | | 2nd | nd | nd | nd | nd |
| | company X | 1st | nd | nd | nd | nd |
| | | 2nd | nd | nd | nd | nd |
| 0 copies/mL | MagDEA Dx SV | 1st | nd | nd | nd | nd |
| | | 2nd | nd | nd | nd | nd |
| | company X | 1st | nd | nd | nd | nd |
| | | 2nd | nd | nd | nd | nd |

**EIC**

| | Extraction reagent | Run | Sputum A | Sputum B | Sputum C | Sputum D |
|---|---|---|---|---|---|---|
| $10^4$ copies/mL | MagDEA Dx SV | 1st | 28.02 | 27.7 | 27.28 | 27.82 |
| | | 2nd | 27.59 | 27.17 | 27.59 | 27.39 |
| | company X | 1st | 26.82 | 26.49 | 25.07 | 24.75 |
| | | 2nd | 26.82 | 25.65 | 26.03 | 25.17 |
| $10^3$ copies/mL | MagDEA Dx SV | 1st | 27.94 | 27.67 | 27.24 | 27.17 |
| | | 2nd | 27.49 | 27.25 | 27.96 | 27.44 |
| | company X | 1st | 27.23 | 26.15 | 24.95 | 25.18 |
| | | 2nd | 26.97 | 27.05 | 25.29 | 25.55 |
| $10^2$ copies/mL | MagDEA Dx SV | 1st | 27.97 | 27.34 | 27.29 | 27.52 |
| | | 2nd | 28.12 | 27.23 | 28 | 27.77 |
| | company X | 1st | 27.59 | 25.61 | 24.73 | 27.14 |
| | | 2nd | 27.63 | 26.91 | 25.24 | 25.16 |
| 0 copies/mL | MagDEA Dx SV | 1st | 28.18 | 28.08 | 27.45 | 27.76 |
| | | 2nd | 27.56 | 26.97 | 27.58 | 27.7 |
| | company X | 1st | 26.55 | 25.69 | 25.12 | 24.55 |
| | | 2nd | 26.22 | 28.14 | 25.07 | 25.59 |

**Fig. 2 : Detection sensitivity test using MTB-added sputum suspensions**

Extraction of QCMD panel samples (MTB2015) was performed. Method B was used for the pretreatment of sputum samples. The results are shown in **Fig. 3**. The MTB detection results from nucleic acids extracted with MagDEA Dx SV and for those extracted with the nucleic acid extraction device of Company X coincided in terms of achieving the expected result.

| | | | Company X | | MagDEA Dx SV | |
|---|---|---|---|---|---|---|
| | Content | Expected result | MTB | EIC | MTB | EIC |
| MTBDNA01 | M. tuberculosis complex (BCG)Frequently deected | Frequently deected | +(30.54) | +(25.98) | +(29.74) | +(29.12) |
| MTBDNA02 | M. xenopi | Negative | nd | +(26.82) | nd | +(28.83) |
| MTBDNA03 | M. tuberculosis complex (BCG)Frequently deected | Frequently deected | +(29.46) | +(24.52) | +(30.5) | +(27.03) |
| MTBDNA04 | M. tuberculosis complex (BCG)Frequently deected | Frequently deected | +(31.29) | +(24.09) | +(33.48) | +(27.48) |
| MTBDNA05 | Mycobaterium negative | Negative | nd | +(24.75) | nd | +(27.62) |

The values in parentheses are the real-time PCR values.

**Fig. 3 : Extraction test using the QCMD panel samples.**

## 〈Conclusion〉

Extraction of *M. tuberculosis* DNA from pretreated sputum and real-time PCR detection were successfully performed with the "MagDEA Dx SV" of PSS.

## 〈References〉

1) Shah and Dye. 1966. Use of Dithiothreitol to Replace N-Acetyl-L-Cysteine for Routine Sputum Digestion-Decontamination for the Culture of Mycobacteria. American Review of Respiratory Disease. 94: 454

## 〈Product information〉

| Product Name | Product No. | Quantity |
|---|---|---|
| MagDEA Dx SV | E1300 | 48 tests |

**Devices**

| Product Name | Product No. | Consumables | | |
|---|---|---|---|---|
| | | Product Name | Product No. | Quantity |
| magLEAD 6gC | A1060 | magLEAD Consumable Kit | F4430 | 50 tests |
| magLEAD 12gC | A1120 | | | |
| geneLEAD XII plus | A2603 | geneLEAD Consumable Set | F2118 | 48 tests |
| | | geneLEAD PCR Cassette | F2121 | 192 tests |
| | | geneLEAD Waste Box | F2125 | 20 items |

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