<Xylene Substitute>

A test was conducted using Hemo-De (FALMA) as a substitute of xylene and confirmed that there was no difference in nucleic acid quality compared to xylene. (**Figures 14, 15, 16, 17, 18**)

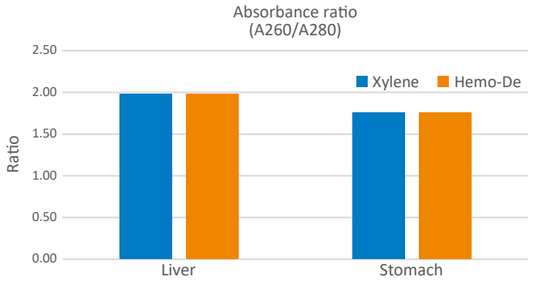

Figure 14. A260/A280 value measured by Nanodrop

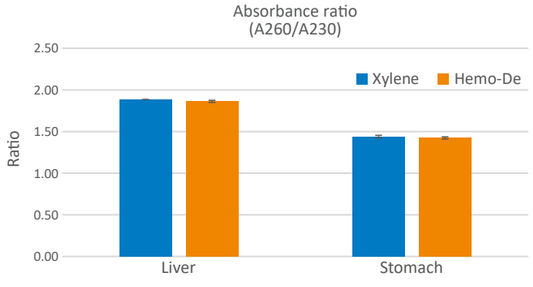

Figure 15. A260/A230 value measured by Nanodrop

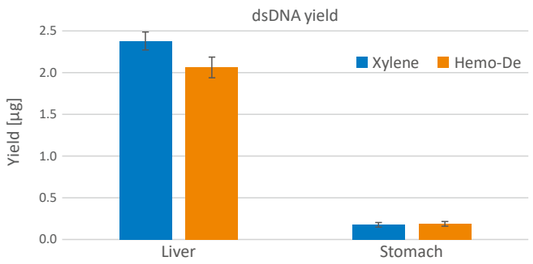

Figure 16. DsDNA yield measured by Qubit dsDNA BR assay

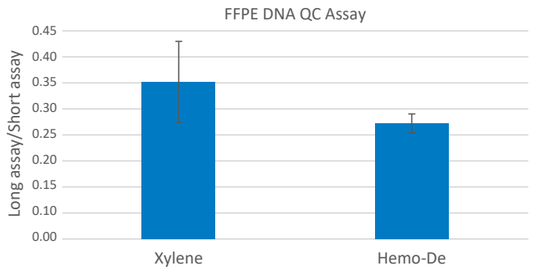

Figure 17. Relative value calculated by FFPE DNA QC assay

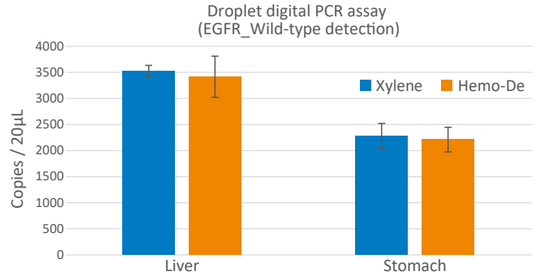

Figure 18. Wild type copy numbers measured by ddPCR assay

〈Conclusion〉

Automated nucleic acid purification from FFPE specimen was possible using the PSS magLEAD system and MagDEA Dx SV (FFPE specimen purification protocol), and nucleic acid purification from micro specimen was also stable. The quality of the purified nucleic acid preparations was equal to or better compared to the Company A spin column method, supporting detection in the molecular diagnostic post-process.

〈References〉

1) Regulations for Handling Pathological Tissue Specimens for Genomic Medicine (The Japan Society of Pathology, 2018)

2) The Japan Lung Cancer Society: Clinical Practice Guidelines for Lung Cancer Using EBM Method 2018

〈Product Information〉

Model	magLEAD 6gC
Reagent	E1300 MagDEA Dx SV
Plastic consumables	F4430 magLEAD Consumable Kit
Protocol (IC card)	MagDEA Dx SV PS
Sample volume	200 µL
Eluent volume	100 µL

Product name	Product code	Contents
MagDEA Dx SV	E1300	48 tests

MagDEA Dx SV Corresponding Device

Product name	Product code	Dedicated consumables		
		Product name	Product code	Contents
magLEAD 6gC	A1060	magLEAD Consumable Kit	F4430	50 tests
magLEAD 12gC	A1120			

Contact details

● For customers in Asia / Pacific
Precision System Science Co., Ltd.
URL: http://www.pss.co.jp
e-mail: service@pss.co.jp

● For customers in North / South America
Precision System Science USA, Inc
URL: http://www.pssbio.com
e-mail: contact@pssbio.com

● For customers in Europe / Africa / Middle East
Precision System Science Europe GmbH
e-mail: contact-psse@pss.co.jp

PSS-TD_E1300_SV200(FFPE)-E-2022.01.Rev001

Nucleic Acid Extraction and Gene Mutation Analysis of Formalin-Fixed Paraffin-Embedded Specimens

(Example usage of fully automated nucleic acid extraction reagent "MagDEA Dx SV (FFPE specimen purification protocol)")

MURATA Yoshihiko
Department of Pathology
University of Tsukuba Hospital

Precision System Science Co., Ltd.

〈Introduction〉

The prevalence of lung cancer shows a gradual upward trend among epithelial malignancies in both men and women. The mortality rate in 2015 was 26.3 % for men, making it the most deadly type of cancer, and 7.6 % for women, corresponding to the cancer type with the third-highest death rate. Although the mortality rates for both men and women have decreased, they are still high compared to other cancers. Between 1998 and 2008, the 5-year survival rate increased from 20.8 % to 27 % for men, 27.1 % to 43.2% for women, and 22.5 % to 31.9 % for men and women combined. Especially among women, there is a massive increase in survival rates compared to cancers affecting other organs. This is mainly due to the increase in tumors that were detected before the onset of metastasis due to the spread of diagnostic imaging, but also due to the extended survival rate of advanced cancers with lymph node and distant metastases. With the development of anti-cancer drugs, the development of personalized therapies tailored to each of various cancer gene metastases such as molecular-targeted drugs and immune checkpoint drugs have had a significant impact.

Searching for various genetic abnormalities – typically performed in preparations of patient DNA or RNA – is indispensable for personalized treatment. As the opportunities for genetic diagnostics using pathological specimens increase, there is a demand for automated systems that enable stable and simple nucleic acid extraction. The magLEAD system (6gC, 12gC) and the nucleic acid extraction reagent MagDEA Dx SV provided by Precision System Science Co., Ltd. (PSS) enable consistent, automated purification of nucleic acids. The instrument is easy to operate, and no special technique is required for setup of the sample to be analyzed, the required consumables, and the reagent cartridge.

In various cancers, analysis of gene mutations/abnormalities is indispensable for realizing personalized treatment, and purified high-quality nucleic acid is required for analysis (1). In the treatment of lung cancer, the results of epidermal growth factor receptor (EGFR) gene mutation test are used to determine the suitability of use of molecular-targeted drug therapeutic agents (2), and FFPE specimens are often used. Nucleic acid purification from formalin-fixed paraffin-embedded (FFPE) specimens is complicated to perform and requires complex techniques to reproducibly obtain high-quality nucleic acid preparations. With the PSS magLEAD system, it is possible to efficiently and reproducibly purify nucleic acids of high quality and high yield.

In genetic diagnosis using FFPE specimens, there are cases where micro specimens are handled and cases where microdissection is performed to increase the rate of introduction of tumor tissue as a method for improving the detection sensitivity of a target gene mutation. Therefore, the ability to reproducibly extract nucleic acids from a sample containing a small amount of tissue is also required. In addition, the purification method should be compatible with substitute products for xylene used in the deparaffinization process for safety reasons.

In this document, we report results of the quality evaluation (Absorbance ratio, dsDNA yield, FFPE DNA QC assay, Agilent DIN value) and the results of the post-process (qPCR, Droplet Digital PCR) for nucleic acids purified from FFPE specimens using the PSS magLEAD system and MagDEA Dx SV reagent (FFPE specimen purification protocol). In addition, we report on the reproducibility between assays, the option to use micro specimens, and xylene substitutes. For comparison with competitor products, a reagent provided by Company A (spin column method) was used for evaluation.

〈Method〉

Lung cancer FFPE specimens (EGFR_L858R_Mutation_5 cases, EGFR_L858R_Wild-type_5 cases) were deparaffinized and decrosslinked using a standard manual method. Nucleic acid purification was performed from treated samples using the magLEAD system and MagDEA Dx SV reagent (FFPE specimen purification protocol) or by using a spin column kit from Competitor A, according to the user manual. The quality verification criteria, post-process evaluation criteria, and test flow of the purified nucleic acid are shown below.
(**Table 1, Figure 1**)

Table 1. Quality verification test criteria, post-process evaluation criteria

Quality Verification Item	Equipment Used
Purity〈A260/A280,260/A230〉	Nanodrop™
dsDNA yield	Qubit® Fluorometer
Fragmentation assessment (DIN value)	TapeStation
Fragmentation assessment (Amplicon length)	7500fast

Post-process Evaluation Item	Equipment Used
Real time PCR (Ct value)	7500fast
Droplet Digital PCRQX200	Droplet Digital PCR system

Figure 1. Testing procedures

<Pretreatment (deparaffinization/decrosslinking) process>

1. Scrape off the tissue section (5 µm thick) on the microscope slide using a change-edge surgical knife and collect the sample in a 1.5 mL tube.
2. Add 1 mL of xylene (a substitute for xylene is also acceptable) to the tube containing the tissue piece and vortex.
3. Centrifuge for 2 minutes at maximum speed (25˚C).
4. Carefully remove the supernatant; take care not to aspirate the pellets.
5. Add 1 mL of ethanol (96-100%) and vortex.
6. Centrifuge for 2 minutes at maximum speed (25˚C).
7. Carefully remove the supernatant; take care not to aspirate the pellets.
8. Leave the tube open for about 10 minutes.
9. Add 180 µL of solution and 20µL of Proteinase K and vortex.
10. Incubate at 56˚C for 1 hour.
11. Incubate at 90˚C for 1 hour.*
    *If there is only one incubator, leave the sample at room temperature while heating it to 90˚C.
12. Spin down the incubated tube and place it in the magLEAD system for nucleic acid extraction.

〈Results〉

<Purity of purified nucleic acids, dsDNA yield>

The purity of nucleic acids purified by the magLEAD system and MagDEA Dx SV reagent (FFPE specimen purification protocol) as judged by absorbance ratios at 260 and 280 nm or 260 and 230 nm, was comparable to that obtained with the Company A spin column method. (**Figure 2, 3**) The yield of dsDNA determined by a Qubit dsDNA BR assay was higher than that obtained with the Company A spin column method (**Figure 4**).

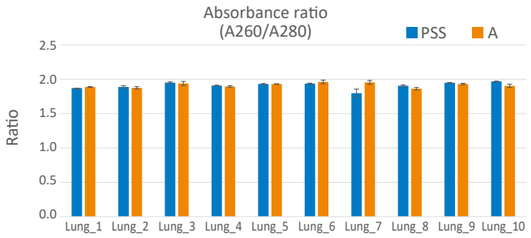

Figure 2. A260/A280 value measured by Nanodrop to evaluate nucleic acid purity

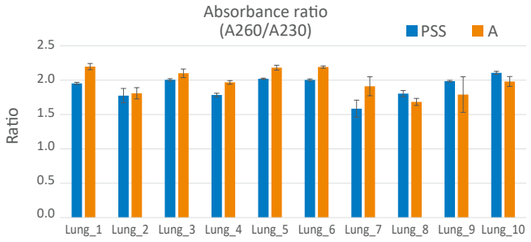

Figure 3. A260/A230 value measured by Nanodrop to evaluate nucleic acid purity

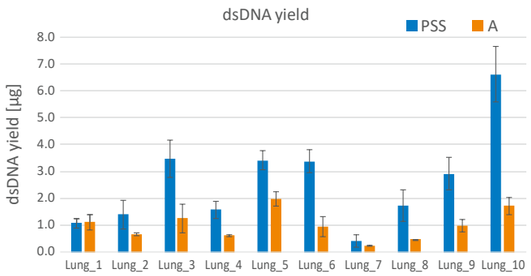

Figure 4. dsDNA yield measured by Qubit dsDNA BR assay

<Value of DIN and FFPE DNA QC Assays>

Good results were shown for the DIN value and the FFPE DNA QC assay of nucleic acid preparations purified with the magLEAD system and MagDEA Dx SV reagent (FFPE specimen purification protocol). The results are equal to or better than those obtained with the Company A kit spin column method (Table 2, Figure 5).

Table 2. DIN value measured by TapeStation

FFPE sample	Automated extraction (PSS)	Spin column extraction (Company A)
Lung_1	2.2	2.2
Lung_2	4.4	3.5
Lung_3	5.9	4.5
Lung_4	6.0	5.4
Lung_5	4.6	3.9
Lung_6	5.4	3.9
Lung_7	5.6	5.0
Lung_8	6.3	5.7
Lung_9	5.7	4.5
Lung_10	4.3	3.3

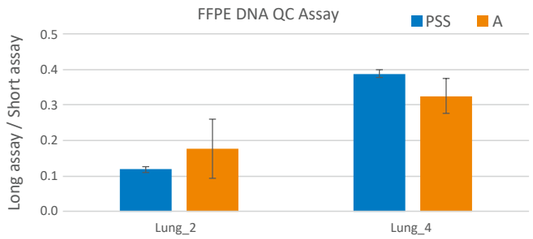

Figure 5. FFPE DNAQC Assay (Thermo Fisher) results (using 7500 Fastsystem)

<Real-time PCR Ct value, Droplet Digital PCR measurement>

When nucleic acids purified by magLEAD system and MagDEA Dx SV reagent (FFPE specimen purification protocol) were detected by real-time PCR, the Ct value was equal to or higher than that of samples obtained with the Company A spin column method (**Figure 6**).

Droplet Digital PCR results were similar to those obtained with the Company A spin column method, and the mutation-positive/negative judgments also matched (**Table 3**).

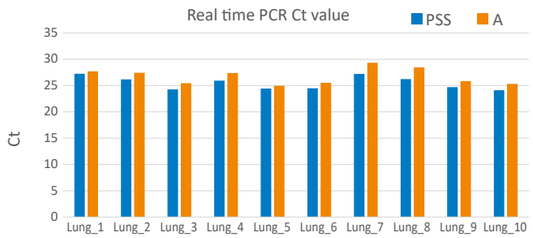

Figure 6. Amplification of the GAPDH gene was confirmed by real-time PCR

Table 3. Mutation positive/negative results by ddPCR assay (mutation content)

FFPE sample	PSS result (L858R %)	Company A result (L858R%)
Lung_1	L858R mutation（21.6 %）	L858R mutation（22.1 %）
Lung_2	L858R mutation（19.8 %）	L858R mutation（19.0 %）
Lung_3	L858R mutation（31.2 %）	L858R mutation（29.9 %）
Lung_4	L858R mutation（13.5 %）	L858R mutation（17.5 %）
Lung_5	L858R mutation（42.1 %）	L858R mutation（41.9 %）
Lung_6	Wild-type（0 %）	Wild-type（0 %）
Lung_7	Wild-type（0 %）	Wild-type（0 %）
Lung_8	Wild-type（0 %）	Wild-type（0 %）
Lung_9	Wild-type（0 %）	Wild-type（0 %）
Lung_10	Wild-type（0 %）	Wild-type（0 %）

<Stability between runs>

Nucleic acid extractions from serial sections of FFPE specimens were performed in 3 different runs (Assay 1, Assay 2, Assay 3). Nucleic acid purity and dsDNA recovery were measured, and mutation detection was performed by digital droplet PCR (**Figure 7, 8, 9**).

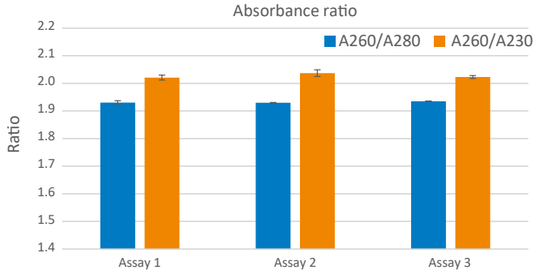

Figure 7. A260/A280 value and A260/A230 value measured by Nanodrop to evaluate nucleic acid purity in 3 independent run

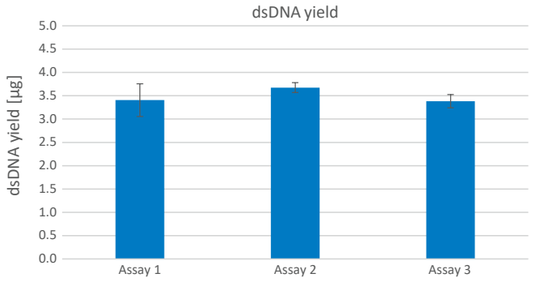

Figure 8. dsDNA yield measured by Qubit dsDNA BR assay in 3 independent runs

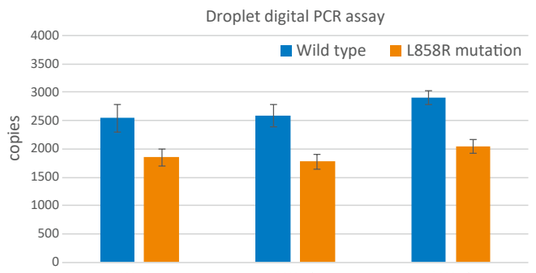

Figure 9. The number of copies of Wild type and L858R mutation measured by ddPCR assay was similar, and there was no difference in the calculated mutation content (42%, 41%, 41%).

<Specimen handling amount and linearity>

FFPE specimen (Lung_5) that has been deparaffinized and decrosslinked was diluted 2-fold, 4-fold, 8-fold, 16-fold, and 32-fold to evaluate whether there is a correlation between the amount of sample handled and the analysis result. Correlation with the amount of sample handled was confirmed in the dsDNA yield and ddPCR measurements (**Figures 10, 11, 12**). When each specimen was analyzed by FFPE DNA QC Assay, no significant difference was observed in fragmentation (**Figure 13**).

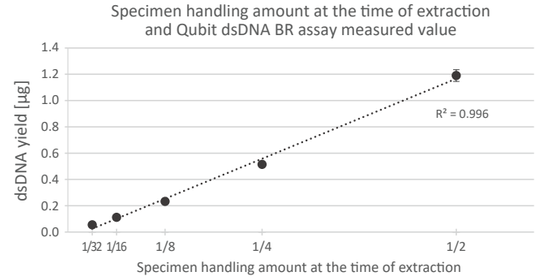

Figure 10. Values fluctuated depending on the correlation between the dsDNA yield measured by the Qubit dsDNA BR assay and the amount of sample handled, and linearity was obtained. dsDNA could be recovered even when a small amount of sample was handled.

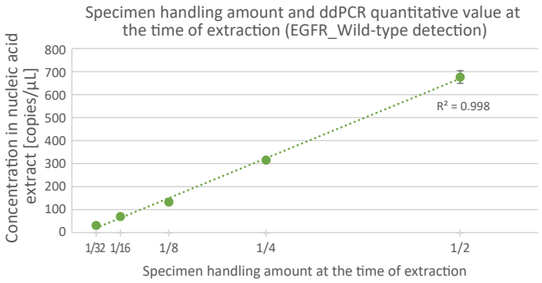

Figure 11. Number of wild type copies in the nucleic acid eluate calculated from ddPCR assay measurements

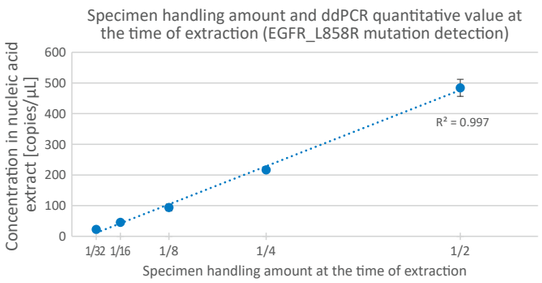

Figure 12:Number of copies of L858R mutation in nucleic acid eluate calculated from ddPCR assay measurements

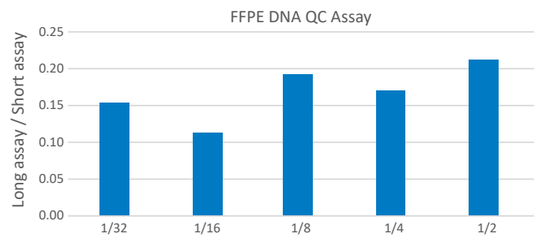

Figure 13: Relative value calculated by FFPE DNA QC assay