DNA extraction from bovine whole blood and leukocyte samples and detection of bovine leukemia virus (BLV)

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

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〈Introduction〉

Bovine leukemia is designated as a notifiable infectious disease under the Act on Domestic Animal Infectious Diseases Control in Japan. Bovine leukemia includes endemic bovine leukemia ("EBL") caused by infection with bovine leukemia virus ("BLV") and sporadic bovine leukemia ("SBL") whose cause is unknown. EBL commonly occurs in cattle aged 4 to 8 years. It causes anorexia, listlessness, exophthalmos, diarrhea, and constipation leading to death in several weeks. Any cattle diagnosed with bovine leukemia at a slaughterhouse are culled in Japan. BLV, which belongs to the Deltaretrovirus genus of the Retroviridae family, infects lymphocytes, integrates its genomic information (genome) into the host bovine genome, and proliferates with host cells. BLV-infected cattle are persistently infected throughout their lives. The infection is horizontally transmitted via horseflies and other bloodsucking insects and blood-contaminated instruments and vertically from a cow to calves. As for the occurrence of bovine leukemia in Japan, the number of BLV-infected cattle had been 200 or less per year from 1998 to 2001 after bovine leukemia was designated as a notifiable infectious disease, but it rapidly increased after 2003 reaching 2,800 in 2015.

It is notable that not all BLV-infected cattle develop EBL: 60 to 70 percent are asymptomatic carriers and approximately 30 percent have persistent lymphocytosis, both of which are clinically normal. Furthermore, since there are no effective vaccines or treatments for EBL, appropriate animal care such as early detection and isolation of BLV-infected cattle is considered an effective prophylactic measure. We believe that "high-risk infected cattle," which highly likely spread infection, can be identified by measuring BLV proviral load (the amount of viral genome integrated into the host bovine genome), and using the resulting measurement as an indicator of transmission risk. Together with appropriate animal care, reduced economic loss and effective infection control would thus be possible.

This article reports the results of DNA extraction from bovine whole blood and leukocyte samples using commercially available extraction kits including a fully automated nucleic acid extraction system, manufactured by Precision System Science Co., Ltd. (PSS), as a pretreatment for measuring BLV proviral load by real-time PCR.

〈Methods〉

Sample

Whole blood and leukocyte samples from BLV antibody-positive Japanese black cattle (*)

* Leukocyte samples were prepared by whole blood hemolysis with a 0.83% NH4Cl and fractioning by centrifugation.

Extraction of DNA from whole blood and leukocyte samples

DNA is extracted from each sample by the methods shown below. The yield and purity (OD260/280) of the extracted DNA were measured with an absorption spectrometer.

Whole blood sample	magLEAD, a fully automated nucleic acid extraction system (PSS), spin column kits (company A, company B)
Leukocyte sample	magLEAD, a fully automated nucleic acid extraction system (PSS), spin column kits (company A, company B), a manual kit (company C)

BLV proviral load measurement

BLV proviral load was quantified by real-time PCR.

〈Results〉

Yield and purity (OD260/280) of extracted DNA

Fig. 1 shows the DNA yield from whole blood and leukocyte samples. **Fig. 2** and **Fig.3** show the purity of DNA extracted from whole blood and leukocyte samples, respectively. The maximum yield was obtained by magLEAD for whole blood samples and by company A's method for leukocyte samples. The purity (OD260/280) of extracted DNA obtained by any of the methods was approximately 1.8 on average.

The purity was analyzed based on the obtained data using one-way analysis of variance (1-way ANOVA) and Tukey's multiple comparison tests **(Fig. 2, Fig. 3)**. The results from whole blood samples showed that there was no significant difference among PSS, company A, and company B. The results from leukocyte samples showed that there was a significant difference between company C and other companies, but the differences among 3 companies (PSS, company A, and company B) were not significant.

	magLEAD	Company A	Company B	Company C
Whole blood	10.8	7.4	7.5	-
Leukocyte	7.9	8.7	7.2	1.0

Fig. 1 : DNA yield from whole blood and leukocyte samples (μg)
(Average value n=25, per 200 μL whole blood)

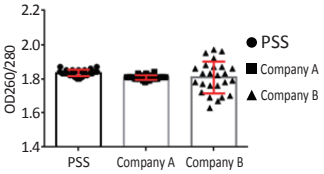

Fig. 2 : Purity of DNA extracted from whole blood samples using each company's extraction method

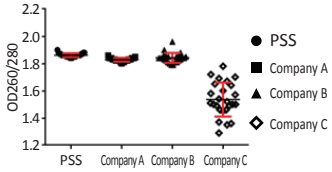

Fig. 3 : Purity of DNA extracted from leukocyte samples using each company's extraction method

Results from quantification of BLV provirus

BLV proviral loads quantified by real-time PCR were analyzed using one-way analysis of variance (1-way ANOVA) and Tukey's multiple comparison tests. **Fig. 4** and **Fig. 5** show the results obtained using the extracted DNA from whole blood and leukocyte samples, respectively. The results from whole blood samples showed that there is no significant difference among the 3 companies (PSS, company A, and company B). The results from leukocyte samples showed that there is a significant difference between company A and company B and between company B and company C.

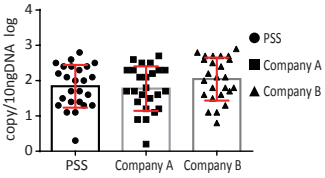

Fig. 4 : BLV proviral loads measured using DNA extracted from whole blood samples

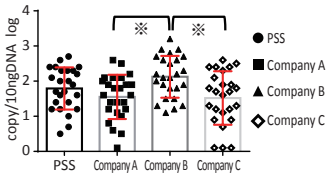

Fig. 5 : BLV proviral loads measured using DNA extracted from leukocyte samples

Comparison of quantified BLV proviral loads

Fig.6 shows the results obtained by one-way analysis of variance (1-way ANOVA) and Tukey's multiple comparison tests for BLV proviral loads quantified using the extracted DNA from whole blood and leukocyte samples. Company A's kit was capable of measuring a larger amount of BLV provirus in whole blood samples while company B's kit was capable of measuring a larger amount of BLV provirus in leukocyte samples. There was not a large difference in the amount detected by PSS's system between whole blood and leukocyte samples. Company A's kit had the smallest variance while company B's kit had the largest variance in measured amount.

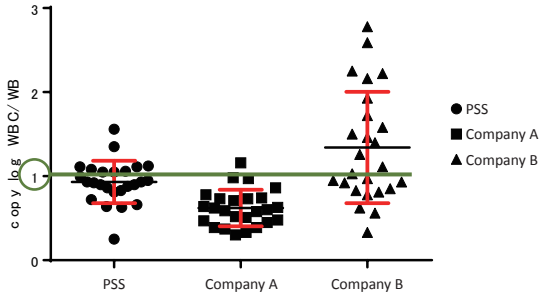

Fig. 6: Comparison of detected amount of BLV provirus using the extracted DNA from whole blood and leukocyte samples

〈Conclusion〉

The performance of PSS's fully automated nucleic acid extraction system, magLEAD, was equal to or better than that of the other companies' spin column or manual kits, and magLEAD was capable of BLV provirus detection. MagLEAD was capable of extracting nucleic acids fully automatically, and the extraction results were more consistent compared to those of manual methods. We may infer that magLEAD is useful in facilitating and effort reduction of testing for the identification of early-stage BLV infection.

〈References〉

1) National Institute of Animal Health, National Agriculture and Food Research Organization (n.d.), Disease information. Retrieved from http://www.naro.affrc.go.jp/niah/disease/

2) Murakami, Kenji (2009), Trends in the occurrence of endemic bovine leukemia and the countermeasures in Japan. J. Vet. Med. Sci. 62: 499-502.

3) Somura, Y., Sugiyama, E., Fujikawa, H., Murakami, K. (2014), Comparison of bovine leukemia virus copies in the lymph nodes of enzootic bovine leukosis cattle and cattle with latent infection. Arch. Virol. 159: 2693-2697.

〈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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