

## Comparison Test of Performance of Proteinase K Stored Cold and Stored Room Temp. Viral DNA Extraction Test

### Objective

To compare the performance of cold-stored Proteinase K (-20°C) and room temperature-stored Proteinase K (25-28°C) used in viral DNA extraction test.

### Passing Criteria

The reading of nucleic acid is detected and correspondence to absorbance value limit for A260 wavelength. Corresponding absorbance value limits for A260 is within the **range of 0.01 to 1.6 Abs** and for **A260/280 is greater than 1.7**.

The amplification of extracted DNA using conventional PCR showed **positive results with 5kb band**.

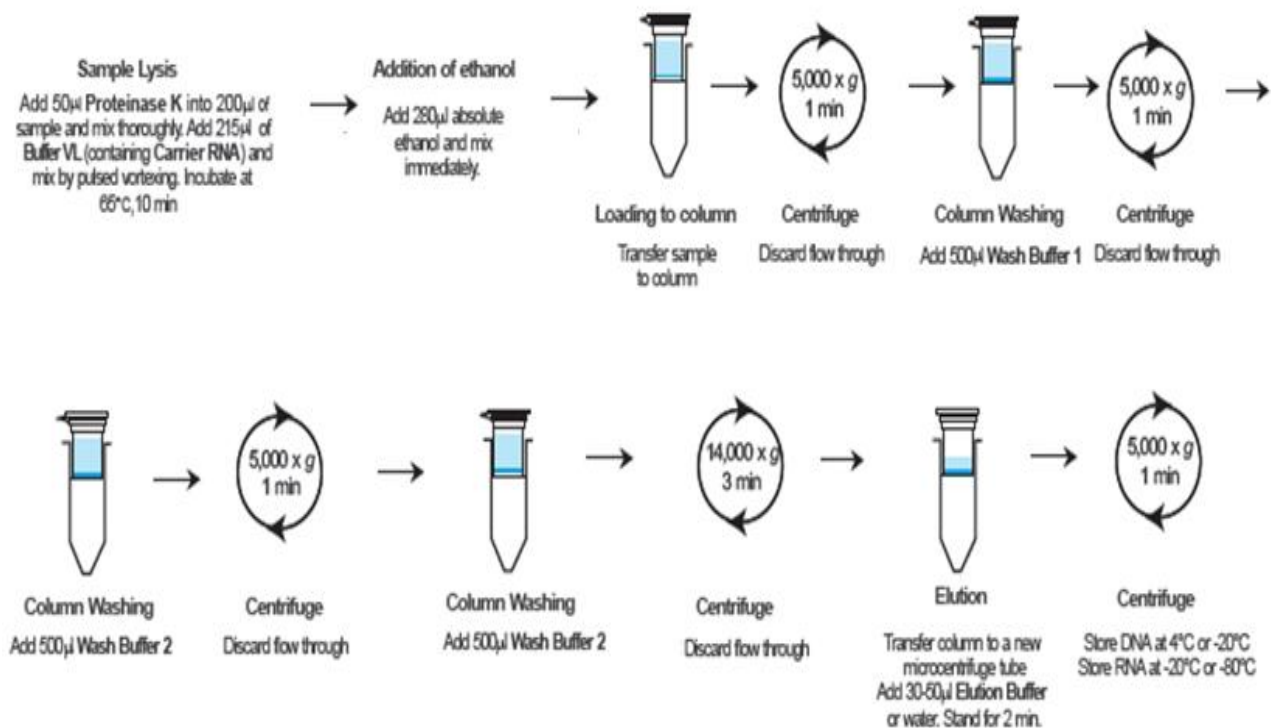
The amplification of extracted DNA using real-time PCR showed positive results with the **difference of Ct value between two Proteinase Ks less than 3**.

### Samples

- Lambda virus spiked into human plasma

### Protocol

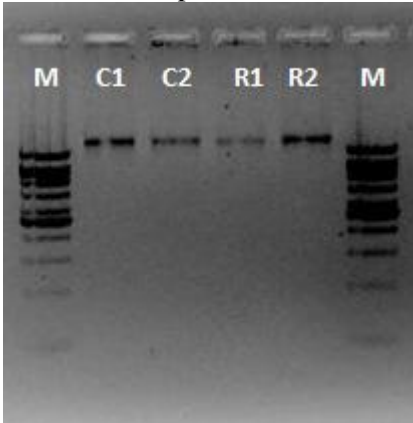
10µl of lambda virus with concentration ±100ng/µl is spiked into 190µl 2X diluted plasma.



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## Results

### Lambda Viral Sample



#### Legend:

C1&C2: Extracted DNA with less than 5ng/ $\mu$ l; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with less than 5ng/ $\mu$ l; extraction using room temperature stored Proteinase K

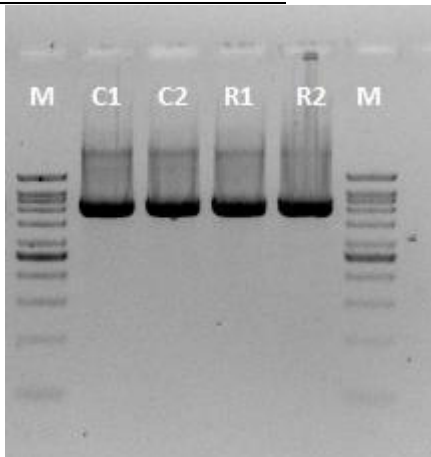
M: VC 1kb DNA ladder

**Figure 1:** 5 $\mu$ l of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

### **Downstream Application**

Conventional PCR and real-time PCR were carried out using the extracted DNA. Both tests were performed using lambda specific primer.

### Conventional PCR Result



#### Legend:

M: 1kb DNA ladder

C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction

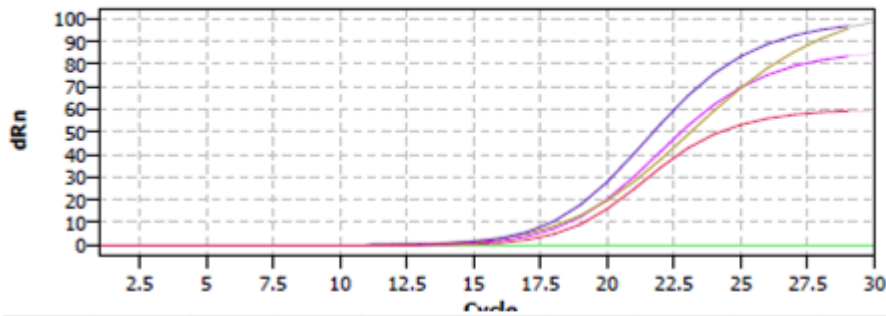
R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction

**Figure 2:** 2 $\mu$ l of extracted DNA was used for amplification. 5 $\mu$ l of PCR product was loaded into 1% TBE gel. The expected band size is 5kb.

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## Real-time PCR Result

GOI



Well	Sample n	Sample t	Gene	Ct	Mean Ct	Conc. St	Mean Co	Std.Dev.	Std.Dev.
F6	RT Lamb	Unknown		21.35	21.35			0	
F5	RT Lamb	Unknown		20.01	20.01			0	
F3	Cool Lam	Unknown		21.01	21.01			0	
F2	Cool Lam	Unknown		20.79	20.79			0	
B4		Unknown		No Ct					

Mean Ct value for RT Lambda	20.680
Mean Ct value for Cool Lambda	20.900
Difference Ct value between RT and Cool	<b>0.220</b>

**Figure 3:** 2µl of extracted DNA was used for real-time amplification. According to the graph and table on top, the difference in Ct value between two different Proteinase Ks is 0.220.

### Conclusion

Lambda viral samples were extracted using GF-1 Viral Nucleic Acid Extraction kit. From the gel photos, there was no significant difference showed in the performance of Proteinase K that was stored in either cold or room temperature condition as the results of amplifications of extracted DNA using conventional PCR showed no significant different for bands; and using real-time PCR showed that all differences between the two Proteinase Ks are within 1Ct value. The sensitivity of the conventional and real-time assay was not affected by the use of room temperature-stored Proteinase K.

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