

Comparison Test of Performance of Proteinase K Stored Cold and Stored Room Temp. Blood 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 blood 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**.

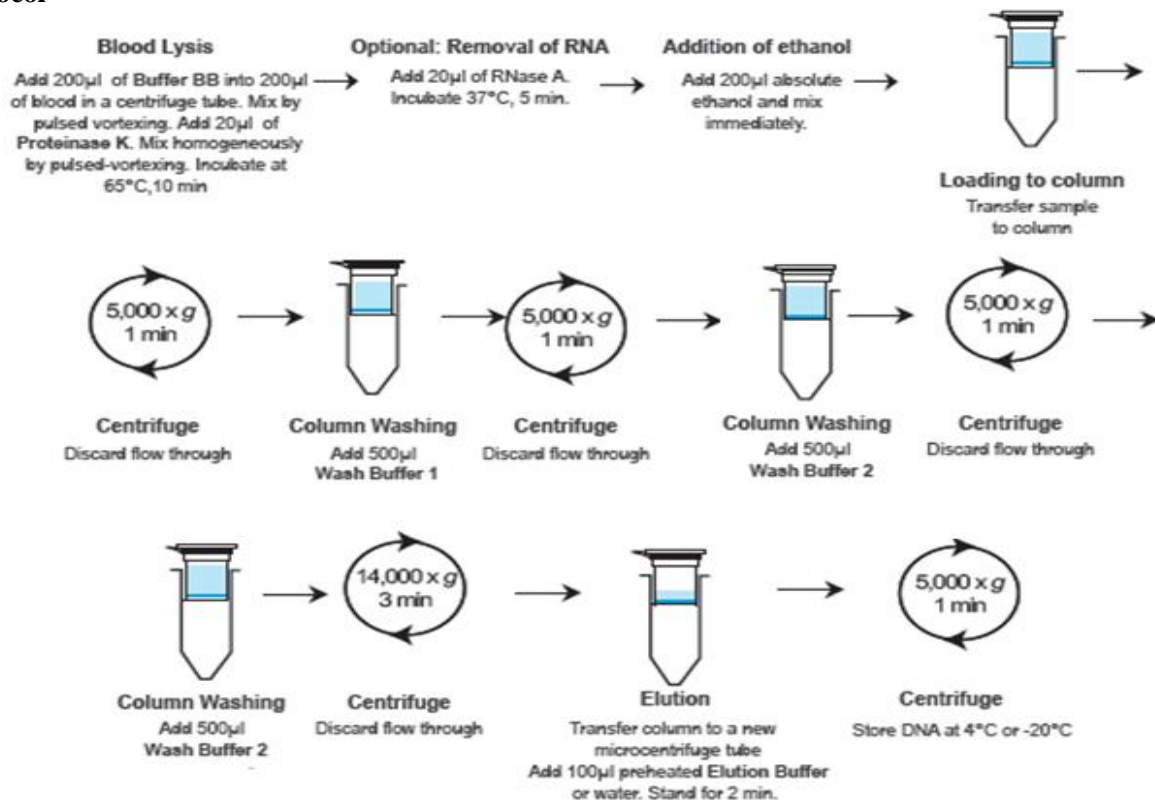
The amplification of extracted DNA using conventional PCR showed **positive results with 350bp band size**.

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

- Frozen blood (blood sample stored up to 1 year)
- Fresh plasma (plasma from whole blood collected fresh)
- Fresh blood (blood sample collected fresh)

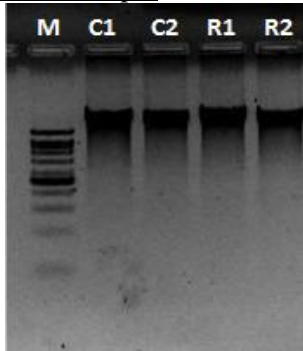
Protocol



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Results

Frozen Blood Sample



Legend:

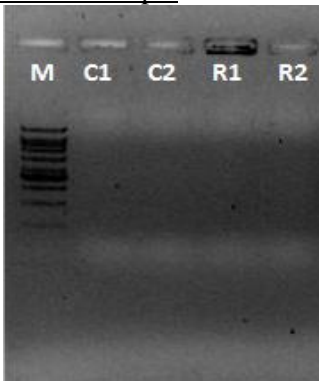
C1&C2: Extracted DNA with more than 50ng/ μ l; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 50ng/ μ l; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

Figure 1: 2 μ l of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Fresh Plasma Sample



Legend:

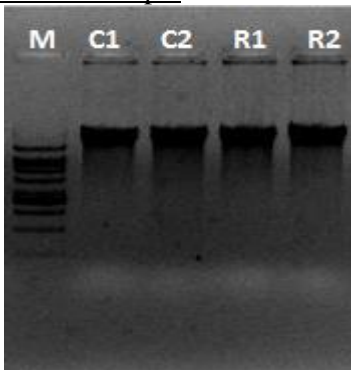
C1&C2: Extracted DNA with less than 10ng/ μ l; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with less than 10ng/ μ l; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

Figure 2: 2 μ l of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Fresh Blood Sample



Legend:

C1&C2: Extracted DNA with more than 50ng/ μ l; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 50ng/ μ l; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

Figure 3: 2 μ l of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

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Downstream Application

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

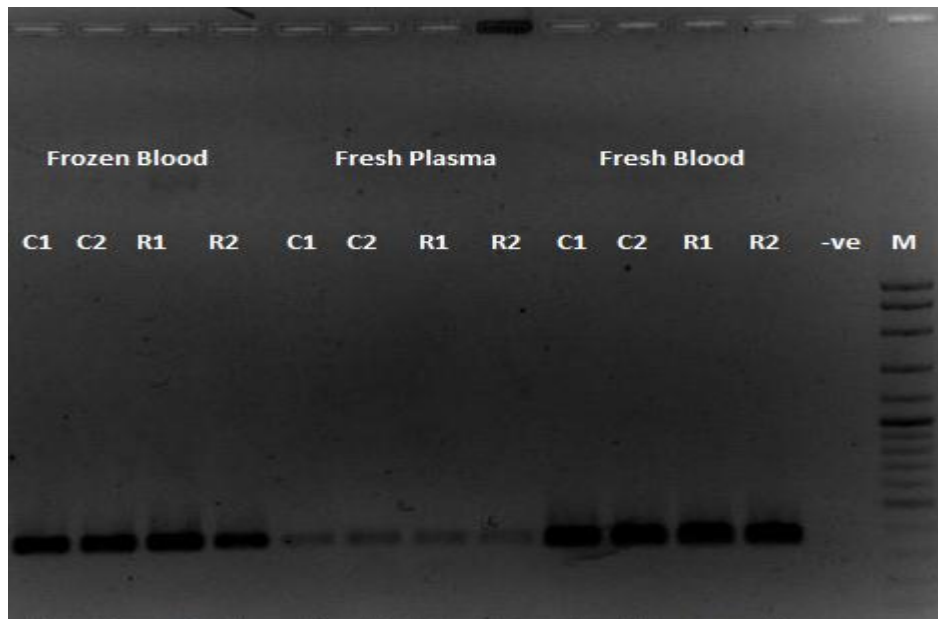


Figure 4: 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.

Legend:

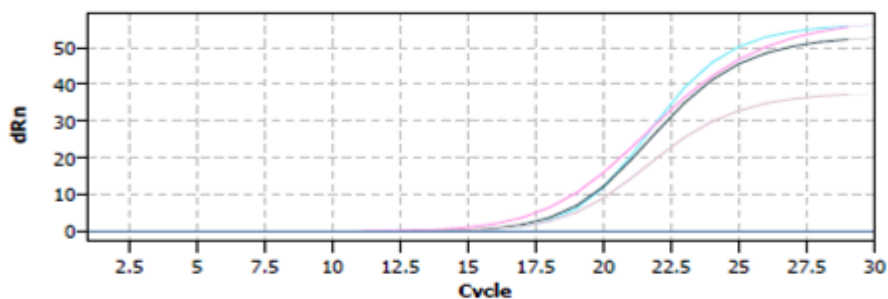
M: 100bp plus DNA ladder

-ve: Amplification product without extracted DNA

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

Frozen Blood Sample



Well	Sample n	Sample t	Gene	Ct	Mean Ct	Conc. St	Mean Co	Std.Dev.	Std.Dev.
G9	RT Froze	Unknown		18.43	18.43			0	
G8	RT Froze	Unknown		17.02	17.02			0	
G7	Cold Froz	Unknown		18.04	18.04			0	
G6	Cold Froz	Unknown		18.26	18.26			0	
G11	-ve	Unknown		No Ct					

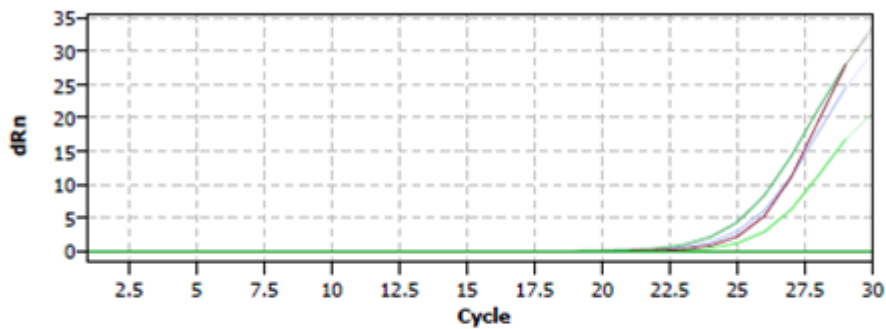
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Mean Ct value for RT frozen blood	17.725
Mean Ct value for Cool frozen blood	18.150
Difference Ct value between RT and Cool	0.425

Figure 5: 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 0.425.

Fresh Plasma Sample

GOI



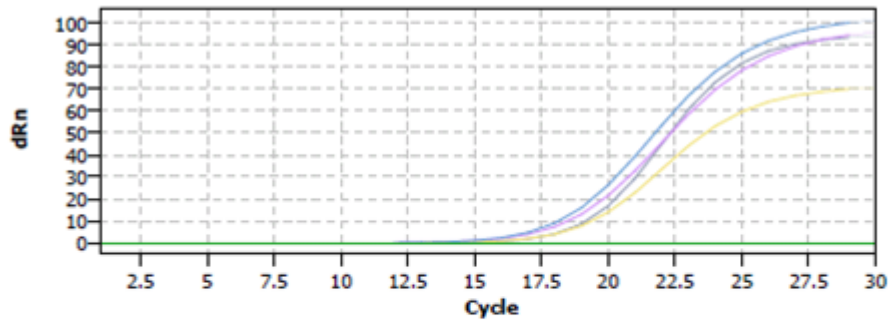
Well	Sample n	Sample t	Gene	Ct	Mean Ct	Conc. Std	Mean Co	Std.Dev.	Std.Dev.
B6	RT Plas	Unknown		25.78	25.78			0	
B5	RT Plas	Unknown		25.06	25.06			0	
B4	Cool Plas	Unknown		26.49	26.49			0	
B3	Cool Plas	Unknown		25.52	25.52			0	
B11	-ve	Unknown		No Ct					

Mean Ct value for RT Plasma	25.420
Mean Ct value for Cool Plasma	26.005
Difference Ct value between RT and Cool	0.585

Figure 6: 2µl of extracted DNA was used for real-time amplification. According to the graph and table showed on top, the difference in Ct value between two different Proteinase Ks is 0.585.

Fresh Blood Sample

GOI



Well	Sample r	Sample t	Gene	Ct	Mean Ct	Conc. St	Mean Co	Std.Dev.	Std.Dev.
B10	RT Blood	Unknown		16.92	16.92			0	
B9	RT Blood	Unknown		18.17	18.17			0	
B8	Cool Blood	Unknown		17.2	17.2			0	
B7	Cool Blood	Unknown		18.12	18.12			0	
B11	-ve	Unknown		No Ct					

Mean Ct value for RT Blood	17.545
Mean Ct value for Cool Blood	17.660
Difference Ct value between RT and Cool	0.115

Figure 7: 2µl of extracted DNA was used for real-time amplification. According to the graph and table showed on top, the difference Ct value between two different Proteinase Ks is 0.115.

Conclusion

3 different samples were extracted using GF-1 Blood DNA 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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