used. Superior detection of miR-16 was obtained using Norgen's modified protocol with a smaller standard deviation. Moreover, Ct values obtained using Norgen's protocol were correlated with the amount of input (i.e. higher input = lower Ct value) indicating the RNA was of high purity and lacked PCR inhibitors. In contrast, the phenol-based method had a higher average Ct value, and did not correlate with input volume, indicating possible PCR inhibition.

Figure 6. Comparison of Relative PCR Inhibition Observed Between Norgen's Total RNA Kit (With Modifications) and the Competitor s Phenol-Based Method for Plasma microRNA Isolation. Total RNA including microRNA was isolated from 200 µL of sheep plasma collected in EDTA tubes and spiked with human microRNAs. Equal portions (4, 8 or 12 µL) of isolated RNA were then used for detection of miR-16 using RT-qPCR.

CONCLUSIONS

From the data presented in this report, the following can be concluded:

1. Norgen's silicon carbide technology provides an efficient and effective way of isolating plasma miRNA without the use of hazardous organic extraction. The resulting advantages includes:
    a. **Better Consistency**
    b. **Better Recovery**
    c. **Faster Protocol**
    d. **High-Throughput (such as 96-wells)**
2. Norgen's silicon carbide technology provides a solution to overcome the main problem associated with plasma miRNA purification - sample source. A consistent microRNA isolation procedure, without the use of phenol is provided for plasma samples collected into various anticoagulants such as Citrate, EDTA and Heparin.
3. **No PCR inhibition** was observed with Norgen's silicon carbide-based plasma microRNA protocol, even for samples purified from blood tubes containing heparin, a known PCR inhibitor.

REFERENCES

1. Carthew, R. W. and E. J. Sontheimer. 2009. Cell. 136: 642-55.
2. Chen, C. et al., 2005. Nucleic Acids Res. 33: e179.
3. Chen, X. et al., 2008. Cell Res. 10: 997-1006.
4. Chim, S. S. et al., 2008. Clin. Chem. 54: 482-90.
5. Flynt, A. S. and E. C. Lai. 2008. Nat. Rev. Genet. 9: 831-42.
6. Gilad, S. et al., 2008. PLoS One. 3: e3148.
7. Hunter, M. P. et al., 2008. PLoS One. 11: 3694.
8. Lawrie, C. H. et al., 2008. J. Haemotol. 141: 672-5.
9. Mitchell, P. S. et al. 2008. PNAS. 105: 10513-8.
10. Raymond et al., 2005. RNA. 11: 1737-44.
11. Schmittgen, T. D. et al., 2008. Methods. 44: 31-8.
12. Taylor, D. D. and C. Gercel-Taylor. 2008. Gynecol. Oncol. 110: 13-21.

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