Effect of RNA Isolation Methods on microRNA Quantity and Quality in Plasma

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ABSTRACT
Small RNAs (miRNAs) are small RNAs that play important roles in gene regulatory networks by binding to and repressing the activity of specific target messenger RNAs (mRNAs). These miRNAs circulate in a highly stable cell-free form within blood and can be detected in plasma and serum. Tumor cells have been shown to release miRNAs to the circulation and profiles of miRNAs in plasma and serum have been found to be altered in cancer and other diseases, suggesting their utility as blood-based markers for molecular diagnostics. Although plasma or serum is an excellent alternative to conventional tissue biopsy, it presents some challenges to miRNA-based diagnostics. In particular, the RNA content in plasma and serum is very low and variable. Hence, effective detection of plasma serum microRNA requires both the RNA purification method and detection method to be sensitive and consistent. While there has been much advancement in miRNA detection technology, the recovery efficiency and quality of miRNA purification has been overlooked. A significant proportion of studies done on plasma or serum miRNA utilized phenol/chloroform extraction. In addition to being lengthy and hazardous, this solvent-based method could result in significant contaminant carryover that could be inhibitory to sensitive downstream applications such as RT-qPCR or microarrays. Here we present data of an evaluation of different sample preparation methods for plasma miRNA. In particular, we compared the common phenol/chloroform/methanol column cleanup method with a silicon carbide-based non-phenol extraction method. Based on a sensitive Locked Nucleic Acid (LNA)-based microRNA quantitation assay we developed, we found that all RNA samples, regardless of the method, were observed to have significant variations in the RNA isolation procedure. This research describes a protocol which overcomes some of these disadvantages for RNA purification from human plasma and serum, as well as, the requirement for data normalization to biologically meaningful results from various forms of gene expression studies due to the carryover of PCR inhibitors into the reaction mixture.

INTRODUCTION
MicroRNAs (miRNAs) are small, non-protein coding RNAs that post-translationally regulate gene expression by suppression of target mRNAs (1 and 2). It has recently been demonstrated that miRNAs circulate within blood in a highly stable, cell-free form and can be detected in plasma and serum (3, 4, 5, 6, 7, 8, 9, 10, and 11). In addition, tumor cells have been shown to release miRNAs into the circulation (12 and 13) and profiles of miRNAs in plasma and serum have been found to be altered in cancer and other diseases (3, 4, 5, 6, 7, 8, 9, and 10). These results indicate the potential for the use of circulating miRNAs as blood-based markers for molecular diagnostics. In order to develop miRNA-based diagnostics the miRNAs must be measured from plasma or serum with sufficient sensitivity and precision to be clinically relevant. The small size of the miRNA allows for sequencing and profiling of miRNAs in plasma and serum that has been found to be altered in cancer and other diseases (3, 4, 5, 6, 7, 8, 9, 10, and 11). These RNA sequences are thus a novel source of information and have been used as biomarkers for various diseases.

miRNAs are widely studied because they are inhibitory to sensitive downstream applications such as RT-qPCR (14 and 15). Microarrays. Here we present data of an evaluation of different microRNA isolation methods. This research describes a protocol which overcomes some of these disadvantages for RNA purification from human plasma and serum, as well as, the requirement for data normalization to biologically meaningful results from various forms of gene expression studies due to the carryover of PCR inhibitors into the reaction mixture.

MATERIALS AND METHODS

A. Blood Collection and Plasma Preparation

Three different species were collected over a six months period, including human plasma collected in EDTA tubes and Heparin tubes to one single entity from each individual. Two individuals were tested. Plasma was prepared according to standard protocol. No additional RNA was spiked in. Hence only endogenous microRNAs were to be detected.

B. Plasma microRNA-Purification Methods

Norgen Total RNA isolation kit (total RNA isolation kit), with modifications of Norgen’s modified protocol for all Citrate and EDTA tubes. In particular, the RNA content in plasma and serum is very low and variable. Hence, effective detection of plasma serum microRNA requires both the RNA purification method and detection method to be sensitive and consistent. While there has been much advancement in miRNA detection technology, the recovery efficiency and quality of miRNA purification has been overlooked. A significant proportion of studies done on plasma or serum miRNA utilized phenol/chloroform extraction. In addition to being lengthy and hazardous, this solvent-based method could result in significant contaminant carryover that could be inhibitory to sensitive downstream applications such as RT-qPCR or microarrays. Here we present data of an evaluation of different sample preparation methods for plasma miRNA. In particular, we compared the common phenol/chloroform/methanol column cleanup method with a silicon carbide-based non-phenol extraction method. Based on a sensitive Locked Nucleic Acid (LNA)-based microRNA quantitation assay we developed, we found that all RNA samples, regardless of the method, were observed to have significant variations in the RNA isolation procedure. This research describes a protocol which overcomes some of these disadvantages for RNA purification from human plasma and serum, as well as, the requirement for data normalization to biologically meaningful results from various forms of gene expression studies due to the carryover of PCR inhibitors into the reaction mixture.

C. microRNA Detection

All microRNAs were converted into cDNA using Exiqon’s miRCURY LNA® Universal RT-qPCR Synthesis Kit (Cuxhaven, Germany) in a portion of purified RNA (4 µl of 50 µl elution) were used in a 20 µl LNA reaction.

The synthesized cDNA was then tested with multiple LNA microRNA primer sets from Exiqon using the Exiqon qScript® RT SuperMix (Bedford, MA, USA) and Exiqon qScript® SYBR Green Master Mix (Cuxhaven, Germany). The resulting qPCR were done in a Bio-Rad Cycler Real-Time PCR system. All qPCRs were carried out in a Bio-Rad Cycler Real-Time PCR system.

Figure 1. Superior Plasma microRNA Recovery from Human Plasma collected from EDTA Blood Tubes by a Silicon Carbide-Based Protocol Without the Use of Organic Extraction. Plasmas were collected from human donors collected into EDTA tubes using Norgen’s Total RNA Purification Kit either with or without modification and compared in a phenol-based method. Isolated RNA was then used for the detection of the various microRNAs using RT-qPCR.

Figure 2. Superior Plasma microRNA Recovery from Human Plasma collected from Citrate Blood Tubes by a Silicon Carbide-Based Protocol Without the Use of Organic Extraction. Plasmas were collected from human donors collected into Citrate tubes using Norgen’s Total RNA Purification Kit either with or without modification and compared in a phenol-based method. Isolated RNA was then used for the detection of the various microRNAs using RT-qPCR.

Figure 3. Superior Plasma microRNA Recovery from Human Plasma collected from Heparin Blood Tubes by a Silicon Carbide-Based Protocol Without the Use of Organic Extraction. Plasmas were collected from human donors collected into Heparin tubes using Norgen’s Total RNA Purification Kit either with or without modification and compared in a phenol-based method. Isolated RNA was then used for the detection of the various microRNAs using RT-qPCR.

DESCRIPTION

1. Plasma miRNA has a huge diagnostic potential including biomarker discovery for various diseases including cancers.

2. Norgen’s silicon carbide technology provides an efficient and effective way of isolating miRNA without the use of hazardous organic extraction. The resulting advantages includes:

   - Better Consistency
   - Better Recovery
   - Faster Protocol
   - Availability for High-Throughput Platforms (such as 96-wells)

3. 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.

4. 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


