New protocols improve detection of microRNAs for diagnosis

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MicroRNAs (miRNAs) that regulate processes including fertilization, development, and aging show promise as biomarkers of disease. They can be collected from routinely collected fluids such as blood, saliva, and urine. However, a number of factors can interfere with the accuracy of miRNA tests. In a study published online today in the *Journal of Molecular Diagnostics*, a group of researchers provide clear procedures for the collection and analysis of miRNA, significantly improving their diagnostic accuracy.

"Our study demonstrates that inherent differences in biological samples and the methods used to collect and analyze them can dramatically affect the detection and quantitation of microRNAs," reports lead investigator Dominik M. Duelli, PhD, Department of Cellular and Molecular Pharmacology, Chicago Medical School at Rosalind Franklin University of Medicine and Science. "We developed measures to overcome the interfering activities and improved the sensitivity of miRNA detection up to 30-fold."

Over 1,000 miRNAs exist in the human body. Deregulation of specific miRNAs is associated with disease. Measuring the amount of miRNAs in body fluids can aid in the diagnosis of disease or conditions such as pregnancy. Dr. Duelli and his colleagues quantified two miRNAs: miR-16, which acts as a tumor suppressor and is deregulated or lost in some cancers, including breast cancer; and miR-223, which has been implicated in pregnancy and other conditions, as well as in some malignant diseases.

"A fundamental challenge to making microRNA diagnostics broadly available has been the inability to isolate enough high quality material to analyze. Our paper outlines ways of effectively collecting blood plasma samples, thus bringing us one step closer to the goal of making [miRNA] disease diagnostics a reality," adds co-investigator Sarah Linnstaedt, PhD, of Duke University.

The authors found that the choice of blood collection tube affects quantitation. Traditional green-top heparin tubes interfered nearly completely with miRNA detection. Grey-top tubes containing the anticoagulant sodium fluoride and potassium oxalate (NaF/KOx) provided the best results. Although miR-16 is about 500 times more abundant in blood plasma than miR-223, the results for both were similar, indicating that the differences in detection resulting from the choice of collection method apply to other miRNAs. Furthermore, collection of miR-223 in serum yielded more variable results, signifying that for some miRNAs, analysis of blood in plasma form is preferred.

The study indicated that natural components of blood plasma co-purify with miRNAs, interfering with their detection. The authors identified extra steps in purification, and the ideal dilution level, to reduce the interference. "Although counterintuitive, by reducing the starting material, inhibitors were presumed to be diluted below a threshold of interference. Careful titration of starting material yields more accurate miRNA quantitation," explains Dr. Duelli. In another approach, the authors avoided the problem of contamination by combining an enzyme that overcomes plasma inhibitors with standard enzymes to increase the sensitivity of miRNA detection by about 30-fold.

Finally, the authors observed that differences in plasma composition among individual donors yield different miRNA measurements. "These results raise the possibility that factors including diet, exercise, circadian rhythms, and seasons, which alter the blood chemistry, might affect

miRNA detection and quantitation," says Dr. Duelli.

"The implications of this work are that without consideration of the variables we have identified, miRNA quantitation of human samples may not be reliable for the purpose of biomarker development. We provide approaches that enable faithful quantitation of miRNA abundance in body fluid," concludes Dr. Duelli.

More information: The article is "Plasma Components Affect Accuracy of Circulating Cancer-Related MicroRNA Quantitation," by D-J. Kim, S. Linnstaedt, J. Palma, J. Cheol Park, E. Ntrivalas, J.Y.H. Kwak-Kim, A. Gilman-Sachs, K. Beaman, M.L. Hastings, J.N. Martin, and D.M. Duelli (doi: 10.1016/j.jmoldx.2011.09.002). Published online ahead of its issue, the study will appear in the *Journal of Molecular Diagnostics*, Volume 14, Issue 1 (January 2012)

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