RNA Testing Now Automated
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From the viewpoint of the clinical laboratory, the consolidation of as many assays and types of assays as possible onto a single and existing automated analyzer (platform) is desirable. Such consolidation, which reduces the complexity and overall cost of a laboratory operation, is exemplified by the implementation of nonseparation (homogeneous) immunoassays such as enzyme multiplied immunoassay technique (EMIT),2 cloned enzyme donor immunoassay (CEDIA), and luminescent oxygen channeling immunoassay (LOCI) onto routine automated clinical chemistry analyzers (1). For relatively complex nucleic acid assays, automation of all or part of the assay has also been accomplished on dedicated analyzers but with relatively limited menus (2). Now there is the prospect of implementing nucleic acid assays on a routine immunoassay analyzer.

An article in this issue of *Clinical Chemistry* addresses the problem of the compatibility of nucleic acid testing with high-throughput clinical laboratory workflows and describes the adaptation of nonamplification microRNA (miRNA) assays to a standard commercial immunoassay analyzer (3). miRNAs are small, noncoding RNAs, 19–25 nucleotides in length, that function as posttranscriptional regulators of up to half of all protein-encoding genes. Their utility as biomarkers derives from their importance in most cellular and developmental processes, and from their remarkable stability, even in body fluids such as plasma, serum, urine, and saliva. Clinical miRNA detection methods include quantitative RT-PCR (qRT-PCR), which is performed routinely in most clinical molecular pathology laboratories, and next-generation RNA sequencing, a highly sensitive (≤fM) and specific methodology, but one that remains costly, in part because of the requirement for extensive bioinformatic capabilities.

The chemiluminescent sandwich assay for miRNA extracted from blood described by Kappel et al. (3) takes <3 h to complete (including the 20-min blood extraction step). On the automated immunoassay analyzer, the blood extract is analyzed in a series of 9 steps, including incubation with biotinylated “catcher” oligo (6 min), coating magnetic microparticles with streptavidin (18-min incubation followed by 6.75-min wash step) and then with an acridinium ester–labeled monoclonal antibody specific for perfectly matched DNA:RNA hetero-hybrids (18 min), and finally, after a wash step, generating a signal from the label in the bound complexes. The detection limit for miRNA (has-miR-5010–3p) was 1 pmol (based on >2SD above median of blank) or 2 pmol (based on signal 2× median of blank) and measured 10% changes in miRNA reliably. The assay also showed concordance with results obtained by qRT-PCR.

Also described in the report are a serial multiplex assay for 8 synthetic miRNAs and a serial multiplex assay for a panel of 4 Alzheimer disease (AD) miRNAs. This somewhat complex stepwise procedure involved serial single-plex assays in which the supernatant from the streptavidin magnetic microparticle step in the assay for the first miRNA was used as the sample in the assay of the second miRNA; this process was repeated for each successive miRNA in the multiplex.

Although this assay represents a significant step forward, several opportunities for improvement remain. The stepwise miRNA assay protocol is much slower than routine simultaneous incubation sandwich immunoassays (e.g., sandwich immunoassays performed on the Advia Centaur® analyzer used in this work only require 7.5-min incubations). The disparity in protocols may make random access immunoassay and RNA testing challenging.

More importantly, and as noted by the authors, their assay is currently insufficiently analytically sensitive to detect miRNAs in serum, precluding its use in the early detection of nonhematologic cancers, for which a number of miRNA profiles are clinically significant (4). Also, the analytical sensitivity and specificity in the multiplex mode are compromised by the different affinities of the anti-DNA:RNA antibody for different DNA:RNA hybrids. This is an important consideration, given that it is usually the patterns of miRNA expression, rather than single miRNA expression levels, that are clinically predictive. For example, using a panel of 12 miRNAs and qRT-PCR, the authors previously showed that the sensitivity and specificity for the detection of AD, compared with healthy controls, were 92% and 95%, respectively (5). Using individuals with neurological disease as

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2 Nonstandard abbreviations: EMIT, enzyme multiplied immunoassay technique; CEDIA, cloned enzyme donor immunoassay; LOCI, luminescent oxygen channeling immunoassay; miRNA, microRNA; qRT-PCR, quantitative RT-PCR; AD, Alzheimer disease.
controls—a clinically more realistic comparison—the sensitivity and specificity of their 12-miRNA panel for AD dropped to 72% and 75%, respectively, suggesting that their panel measures, at least in part, neurological disease generally, and not just AD per se. In the present study, they used a 4-miRNA subset of their 12-miRNA panel and found a sensitivity and specificity for the detection of AD compared with healthy volunteers of 80% and 85%, respectively; to achieve a positive predictive value of, for example, 85%, with this sensitivity and specificity, the prior probability of AD in the population analyzed would already have to be >50%. Larger miRNA panels, such as their 12-miRNA panel, generally improve predictive values, which favors microarray technologies (or qRT-PCR).

Nevertheless, the assay has many positive features. The equipment needed is already installed in hospitals worldwide, no amplification is needed, and it should be possible to adapt the assay to other RNA targets. Microarray technology has a similar analytical sensitivity, but it is complex and expensive for clinical laboratories. As mentioned above, next-generation RNA sequencing has superior performance characteristics, but also remains complex and expensive to implement. At present, the real competition for the assay described herein is qRT-PCR, which, because it involves amplification, is sufficiently analytically sensitive for the detection of cell-free miRNAs. However, for the detection of expression patterns of cell-derived miRNAs, such those found in hematologic malignancies (and solid tumors, when tumor tissue is available), the speed, simplicity, and workflow advantages of the assay described herein could make it a very important addition to the clinical laboratory armamentarium.

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