Reviewer's report

Title: A Novel Multiplex Polymerase Chain Reaction Assay for Profile Analyses of Gene Expression in Peripheral Blood

Version: 2 Date: 9 April 2012

Reviewer: James Wingrove

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Major Compulsory Revisions

While the technical aspects of assay development and performance measurement in the manuscript appear to be fairly solid and I appreciate the improvements the authors have made in the manuscript (e.g. addition of the replication set), I feel the clinical piece is less well developed and in some cases incorrect (e.g. the statement “Atherosclerosis is not considered important until a myocardial ischemia occurs”; first paragraph in Background, page 3, should be removed).

The authors have now included an ROC analysis (Table 4) and conclude that the 4 gene model is a better classifier than any of the single genes alone. While the AUC is higher for the 4 gene model, in order to substantiate this claim the authors need to demonstrate this increase is significant (as well as provide 95% confidence intervals). What threshold was used to determine sensitivity and specificity? Was the threshold prospectively defined? What was the performance of the model in the replication set of subjects?

The AUC for the 4 gene model is modest at best, with poor sensitivity leading one to wonder about the clinical utility of the model; does it perform better than clinical factors in identifying patients with non-calcified plaque in this population? How does it compare to published diagnostic models using clinical factors (e.g. Diamond-Forrester)? How would such a model be used clinically? The higher specificity suggests as a rule-in test; what is the clinical action associated with the result?

Unless the authors can substantially improve the manuscript in this area, I feel this portion should be removed, and the primary emphasis focused on the technical aspects of assay development and performance.

Additionally, despite improvements the manuscript still needs editing for grammar.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

Employee of CardioDx, Inc.