DEVELOPMENT OF A BIOCHIP ARRAY FOR THE DETECTION OF ADHESION MOLECULES ON THE NEW RANDOM ACCESS FULLY AUTOMATED EVIDENCE EVOLUTION ANALYSER

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INTRODUCTION

Cell adhesion molecules are complex membrane proteins which mediate cell-to-cell interactions and subsequently influence a wide range of intracellular signalling cascades. Adhesion molecules can also be detected in soluble forms in the circulation. These molecules are implicated in a diverse range of physiological processes such as cell proliferation, migration, differentiation, apoptosis, and the mediation of inflammatory processes. Altered circulating levels of adhesion molecules have been reported in a wide range of physiological conditions, such as cardiovascular disease, stroke, cancer, chronic kidney disease (CKD) and diabetes. Consequently, the measurement of circulating adhesion molecules has importance for identifying and monitoring disease.

The objective of this study is to utilize Randox's proprietary, multiplexing biochip array technology to develop an Adhesion Molecule array for application to the new, fully-automated Evidence Evolution analyser. The array encompasses five adhesion molecules – Vascular Cell Adhesion Molecule 1 (VCAM-1), Intracellular Adhesion Molecule 1 (ICAM-1), E-selectin (ESEL), P-selectin (PSEL) and L-selectin (LSEL).

METHODOLOGY

Antibodies specific to VCAM-1, ICAM-1, ESEL, PSEL and LSEL were immobilised to discrete testing regions within a biochip surface and a chemiluminescent sandwich immunoassay format was used for this array. The array has been developed for application to the Evidence Evolution analyser which requires minimal user input and provides rapid results, with the first test read after 36 minutes and a test per minute thereafter. Assay sensitivity, precision, cross-reactivity and interference were evaluated to define assay characteristics. Clinical utility was also evaluated using a cohort of 41 samples (22 healthy controls and 19 CKD).

RESULTS

Cross-reactivity analysis demonstrated that each individual assay was specific for its target analyte and that no cross-reactivity was observed with non-panel homologous proteins (cross-reactivity <1%). Common blood interferents - haemoglobin, triglycerides, intralipids and bilirubin – demonstrated no interference with assay performance.

The assays were simultaneously evaluated and yielded the following ranges and sensitivities:

Assay	Sensitivity (ng/mL)	Range (ng/mL)
VCAM-1	34	0-5658
ICAM-1	13	0-1513
ESEL	4	0-396
PSEL	29	0-2328
LSEL	36	0-6212

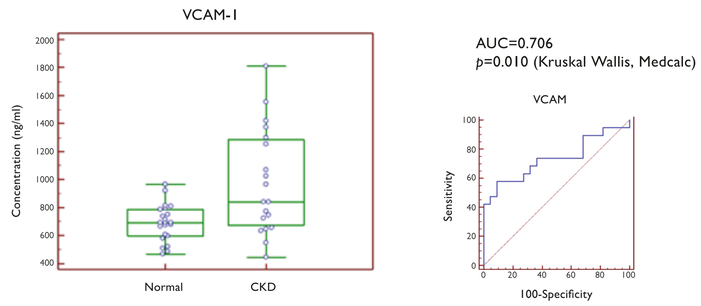
17.096, 097.105RDRT

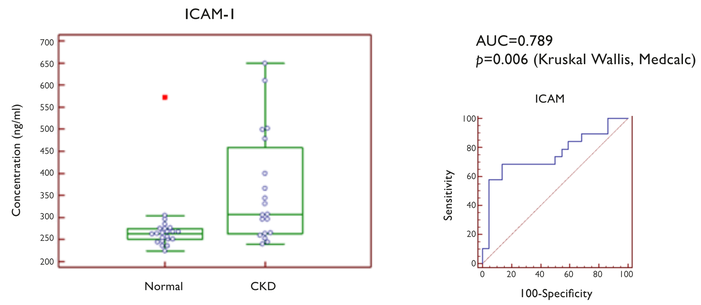
Repeatability and within-laboratory precision were assessed (n=80):

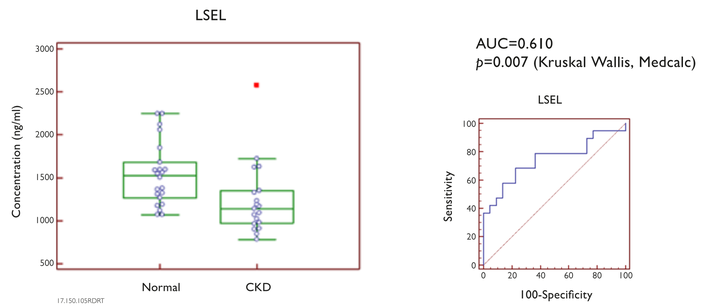
Assay	Repeatability	Within-laboratory precision
	CV (%)	CV (%)
VCAM-1	4.5	6.8
ICAM-1	5.6	8.8
ESEL	12.8	16.6
PSEL	3.5	4.7
LSEL	5.7	8.2

17.096, 097.105RDRT

Clinical utility was evaluated using a cohort of 41 samples (22 healthy controls and 19 CKD). Significant differences in biomarker concentrations were observed when CKD samples were compared to controls - VCAM-1 (AUC = 0.706; p = 0.010), ICAM-1 (AUC = 0.789; p = 0.006) and LSEL (AUC = 0.610; p = 0.007). No significant differences were observed for ESEL and PSEL.







17.150.105RDRT

CONCLUSION

This study reports on the development of a multiplexed array for the simultaneous measurement of VCAM-1, ICAM-1, ESEL, PSEL and LSEL, applied to the new fully automated Evidence Evolution analyser. This array offers a rapid, fully-automated alternative to traditional ELISA methods, with minimal sample volume requirements. This newly developed Adhesion Molecules array can be applied to a diverse range of pathologies.