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LONG-TERM QUALITY CONTROL OF THE CYTOKINE & GROWTH FACTORS AND CELL ADHESION MOLECULE ARRAYS AT THE RANDOX EVIDENCE INVESTIGATOR

DUGOTRAJNA KONTROLA KVALITETA CITOKINA, FAKTORA RASTA I ĆELIJSKI ADHEZIONIH MOLEKULA NA RANDOX EVIDENSU

Johannes van Pelt, Fred P. H. T. M. Romijn

Department of Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands

Summary: Multi component assays are a promising development in laboratory medicine. Reproducibility and standardization of the used technology is crucial for the quality of the results. In our laboratory several studies were carried out in a period of more than two years using the Randox Evidence Investigator. We applied the Cytokine & Growth Factors and Cell Adhesion Molecule Array from which reference values could be obtained. Cytokines are not useful in low grade inflammation or in normal persons as the levels are too low to detect. However growth factors and cell adhesion molecules could be determined in those studies. Outcomes with the arrays were compared with conventional assays such as ELISA. The correlations of IL-6 and IL-10 were very good and that of s-ICAM acceptable. Inter-assay coefficients of variation could be calculated by using the same control level material during those years. The system turned out to be easy to handle and very stable over a long period of time with CV's of about 8–12%. The results obtained are not dependent of the lot number of the arrays or apparatus as two different apparatus gave same outcomes.

Keywords: biochip array, multi component analysis, quality control

Kratak sadržaj: Multikomponentna određivanja su veoma obećavajuća u laboratorijskoj medicini. Reproducibilnost i standardizacija primenjene tehnologije je veoma značajna za kvalitet rezultata. U našoj laboratoriji izvedeno je nekoliko proučavanja u periodu od preko dve godine primenom Randox Evidence Investigator-a. Izučavani su citokini, faktori rasta i ćelijski adhezioni molekuli primenom array tehnike za koje je bilo moguće dobiti referentne vrednosti. Citokini nisu korisni u slučaju inflamacije niskog stepena ili kod zdravih osoba s obzirom da su im nivoi veoma niski. Međutim faktori rasta i ćelijski adhezioni molekuli su izučavani u ovim studijama. Dobijene vrednosti su poređene primenom konvencionalne ELISA tehnike. Dobijene su veoma dobre korelacije za IL-6 i IL-10, a za s-ICAM prijhvatljive. Koficijenti varijacije između određivanja su izračunati za kontrolu istog nivoa u toku period ispitivanja. Pokazalo se da je sistem veoma stabilan i pogodan za određivanja u dugom periodu sa koeficijentima varijacije od oko 8–12%. Dobijeni rezultati nisu zavisili od lota reagensa ili aparata, s obzirom da su primenjiva dva aparata u toku ispitivanja.

Ključne reči: biočip array, multikomponentna analiza, kontrola kvaliteta

Introduction

The field of clinical proteomics is growing and several new technologies have become available for 'routine' use in clinical chemistry laboratories. Simultaneous measurement of multiple markers is now possible with biochip array or multiplex technology (1–3). Multicomponent assays are a promising development in laboratory medicine especially in exploratory studies or in case of first 'screening'. Application of such arrays can lead to the discovery of new protein markers for diagnosis, prognosis or therapeutic efficacy and will give more insight in the complex interplay of proteins in health and disease (4, 5). Reproducibility and standardization of the biochips is crucial for the quality of the results as measurements are frequently carried out during a long period (6, 7). Analytical accuracy and quality of data are the basis for the quality of the generated clinical findings.

In our laboratory several studies were performed in a period of about two years with the Cytokine & Growth Factors and Cell Adhesion Molecule Array

Address for correspondence:

Johannes van Pelt
Department of Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands

using the Randox Evidence Investigator. From those studies reference values could be obtained. Furthermore, the cumulative results of the control levels give an excellent view of the long-term stability of the system and the applied biochip technology.

Methods

The cytokines, growth factors and adhesion molecules were measured using a Randox Evidence Investigator and the Cytokine & Growth Factors Array and Adhesion Molecules Array. With the Evidence Investigator Biochip Array Technology it is possible to perform simultaneous quantitative detection of multiple analytes in a single sample of 20 or 100 µL serum. The sandwich chemiluminescent immunoassay technology makes use of a Randox Biochip containing an array of discrete test regions of immobilized antibodies specific to IL-1α, IL-1β, Il-2, IL-4, IL-6, IL-8, IL-10, IFNγ, TNFα, VEGF, MCP-1 and EGF in case of Cytokine & Growth Factors Array and soluble E-, L- and P-selectin, ICAM-1 and VCAM-1 in case of Adhesion Molecules Array. The antibodies are immobilized in regions of 9 mm^2 in an ordered array arrangement. The biochips are supplied in carriers containing 3 × 3 biochips and a carrier handling tray allows simultaneous handling of 6 carriers e.g. 54 biochips. The light signal generated from each test region on the Biochip with antibodies labeled with Horse Radish Peroxidase is detected using a super-cooled charge coupled device camera and compared to that from a stored calibration curve.

In short, the sample is diluted with assay buffer or diluent and applied to a biochip (well). The biochip (carrier) is incubated at 37 °C and shaken at 370 rpm in a thermoshaker for 60 min. After washing the conjugate (HRM labeled antibodies) is added and again incubated at 37 °C and shaken at 370 rpm in a thermoshaker for 60 min. After washing 250 µL of a 1 : 1 mix of luminol and peroxide is added and incubated for 2 min. Finally the carrier is imaged using an Investigator System with digital imaging technology.

Results and Discussion

Cytokines are not useful in low grade inflammation or in normal persons as the levels are too low to detect and mostly below the sensitivity of the assay. However, growth factors and cell adhesion molecules could be determined in those studies and gave valuable information (data not shown). Outcomes with the arrays were compared with conventional assays such as ELISA. The correlations of IL-6 and IL-10 were very good over a wide clinical relevant range (see *Figure 1*) with a correlation coefficient of 0.93 and 0.97 respectively. The values however were different from those obtained by microtiter plate methods. Inter-assay coefficients of variation could be calculated by using the same control level material

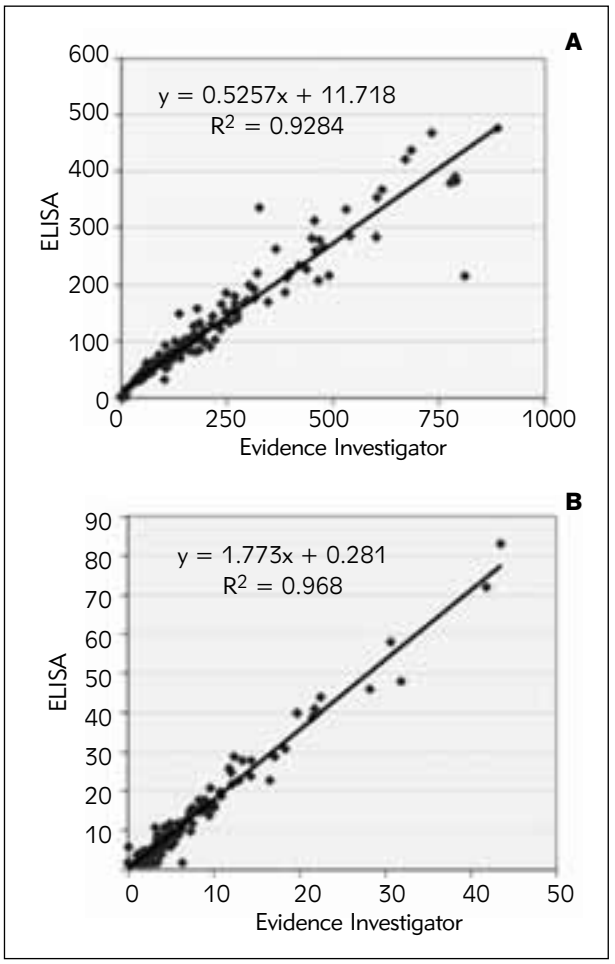

Figure 1 Results of correlation studies of IL-6 (A) and IL-10 (B) in pg/mL obtained with the Evidence Investigator in comparison with conventional ELISA methods.

during a long period. In *Table I* the results are presented of 3 control levels from the Cytokine and Growth Factors array which have been measured 15 times during two years. Those data show a coefficient of variation of around 8–12%. In *Table II* the results are presented of 3 control levels from the Cell Adhesion Molecule array which have also been measured 15 times during the same period. The controls had different lot numbers which are indicated but the outcomes are comparable and the mean coefficient of variation is also around 10%. As we used two different apparatuses during this period we were able to compare the outcomes but no difference was observed in the results (data not shown). The impact of a stable quality control system is visualized in *Figure 2* which shows the outcomes of the IL-10 measurements in a control sample with two different lot numbers during a period of two years with and without preceding calibration.

The reproducibility and long-term stability are essential for measurements in the multicomponent field using new technologies like biochip array

Table I Performance of Cytokine & Growth Factors Array on Evidence Investigator in pg/mL. Inter-assay precision over 2 years (n=15).

	Level 1			Level 2			Level 3		
	Conc	SD	%CV	Conc	SD	%CV	Conc	SD	%CV
IL-2	35.8	3.4	9.4	193.2	11.8	6.1	565.1	54.3	9.6
IL-4	37.2	5.4	14.4	245.6	27.0	11.0	634.4	54.1	8.5
IL-6	4.6	0.5	11.3	43.2	3.3	7.7	90.9	10.6	11.6
IL-8	41.5	4.5	10.9	307.6	23.0	7.5	1206.6	158.2	13.1
IL-10	17.3	1.5	8.6	205.0	17.5	8.5	518.3	57.2	11.0
VEGF	31.9	3.7	11.5	389.5	47.5	12.2	718.5	50.6	7.0
IFNγ	8.1	1.6	19.3	208.6	19.5	9.4	795.9	127.8	16.1
TNFα	16.2	1.6	9.8	204.6	9.5	4.6	704.1	68.8	9.8
IL-1α	6.4	0.6	9.7	94.2	9.4	10.0	297.0	32.3	10.9
IL-1β	9.1	1.2	13.3	130.5	14.4	11.1	>225		
MCP-1	35.5	6.3	17.8	185.7	17.2	9.2	412.8	43.3	10.5
EGF				87.5	7.3	8.3	305.1	16.9	5.6

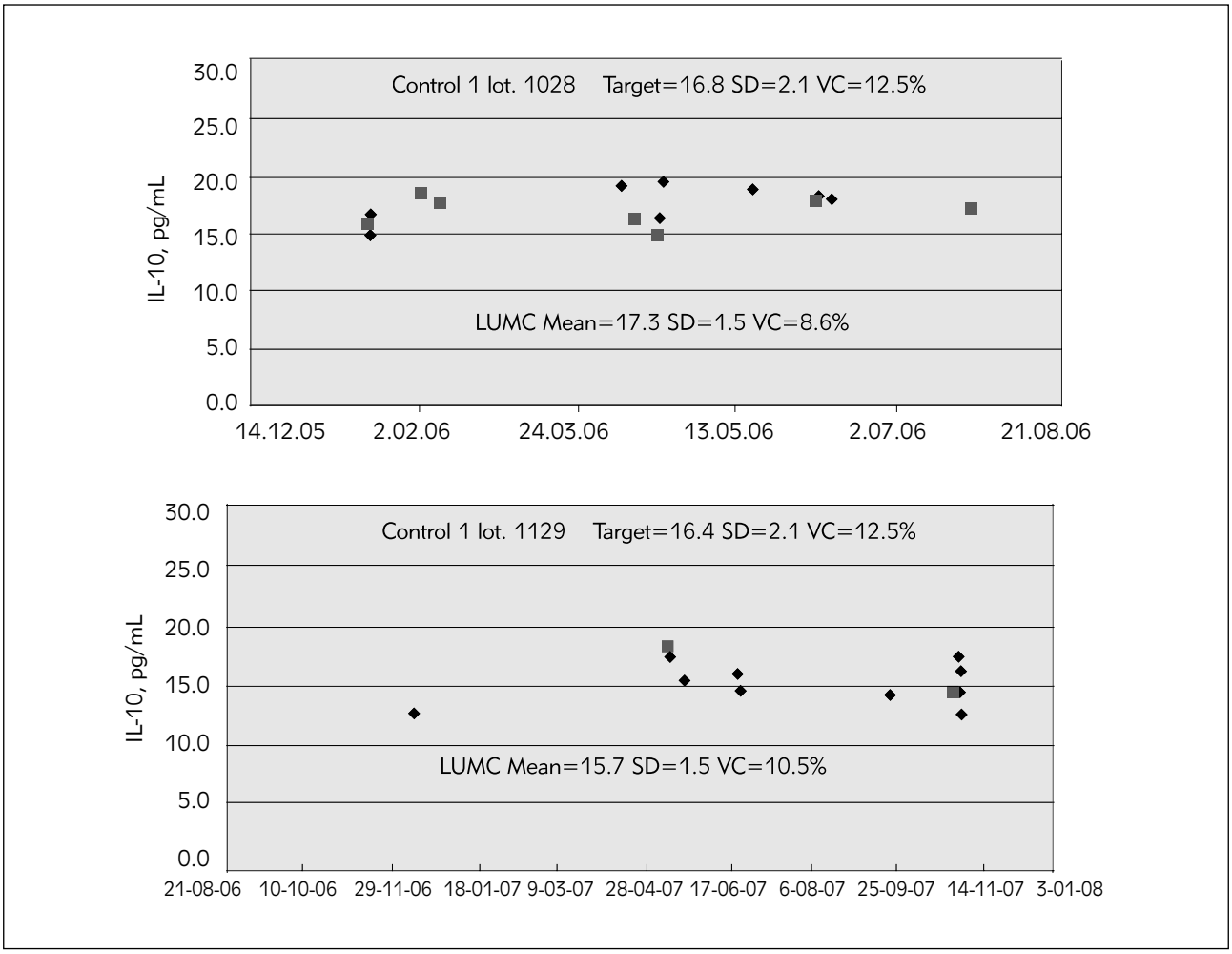

Figure 2 Results of measurement of IL-10 in two quality control samples, lot 1028 (A) and lot 1129 (B), during two years. The squares are results with calibration and the diamonds are results without calibration.

Table II Performance of Adhesion Molecules Array on Evidence Investigator. Inter-assay precision over 2 years. The left columns of the concentration and the SD are the experimental results and the right columns represent the target values. For the %CV the target value is always 12.5%.

Analyte	lotnr. 1015					lotnr. 1223					lotnr. 1478				
	Conc (ng/mL)		SD		%CV	Conc (ng/mL)		SD		%CV	Conc (ng/mL)		SD		%CV
Level 1															
VCAM-1	21.77	20.63	3.51	2.58	16.1	24.62	23.69	1.44	2.96	5.8	22.24	23.80	4.02	3.00	18.1
ICAM-1	5.38	6.25	0.51	0.78	9.5	6.34	5.63	1.07	0.70	16.8	6.31	5.67	0.31	0.71	4.8
E-Selectin	1.41	1.56	0.24	0.20	17.3	1.08	1.14	0.13	0.14	12.1	1.10	1.15	0.12	0.14	10.7
P-Selectin	8.47	7.50	0.58	0.94	6.9	6.74	6.72	0.39	0.84	5.8	7.11	6.98	0.30	0.87	4.2
L-Selectin	23.35	21.88	2.29	2.74	9.8	26.92	23.58	1.59	2.95	5.9	25.88	25.20	2.54	3.20	9.8
Level 2															
VCAM-1	38.69	41.25	4.22	5.16	10.9	44.08	42.28	4.68	5.29	10.6	47.95	44.10	1.32	5.50	2.8
ICAM-1	10.90	12.50	1.26	1.56	11.6	12.10	10.15	0.98	1.27	8.1	11.84	11.00	0.58	1.40	4.9
E-Selectin	2.51	3.13	0.19	0.39	7.8	2.19	2.22	0.21	0.28	9.4	2.44	2.27	0.29	0.28	12.0
P-Selectin	15.17	15.00	0.83	1.88	5.4	13.35	12.73	1.10	1.59	8.2	13.31	13.20	0.83	1.70	6.2
L-Selectin	42.42	43.75	2.57	5.47	6.1	50.65	47.11	2.47	5.89	4.9	49.50	49.50	1.24	6.20	2.5
Level 3															
VCAM-1	65.43	82.50	3.75	10.31	5.7	78.65	76.71	10.0	9.59	12.7	91.50	81.20	2.68	10.20	2.9
ICAM-1	22.80	25.00	1.36	3.13	6.0	26.47	22.70	1.84	2.84	7.0	26.71	24.50	3.24	3.10	12.1
E-Selectin	5.62	6.25	0.31	0.78	5.6	5.19	4.62	0.83	0.58	15.9	5.11	4.83	0.46	0.60	9.0
P-Selectin	28.03	30.00	1.67	3.75	5.9	25.38	25.79	2.08	3.22	8.2	25.61	26.10	1.51	3.30	5.9
L-Selectin	84.73	87.50	9.47	10.94	11.2	96.88	93.26	12.03	11.66	12.4	90.28	97.60	10.20	12.20	10.2

methodology. Good controls are crucial as they are the most important tool to determine if the results from one experiment are comparable with those of another experiment.

The system turned out to be easy to handle and very stable over a long period of time with mean coefficients of variation of around 10% which is comparable with those published earlier for the Cytokine and Growth Factors array (2) and recently for the Cell Adhesion Molecule array (8).

The CV's are in the same range as conventional ELISA based microtiter plate methods. The results obtained are not dependent on the lot number of the arrays or apparatus as two different apparatuses gave the same outcomes. It was not necessary to have frequent calibration and the standard curves turned out to be very stable.

One major advantage of the Evidence biochip array technology is time saving. In one day two runs of 54 samples can be performed easily yielding about 700 results. Applying 12 different ELISA based assays will take much more time. The other important advantage is the amount of material needed. In all biochip arrays only 20–100 μL of material is used while conventional assays demand sometimes 2 × 100 μL per determination.

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