Metabolik Hastalıkların Multiplex Sistemlerle Analizi

Yrd.Doç. Dr. Türkan Yiğitbaşı

Katip Çelebi Üniversitesi Tıp Fakültesi
Biyokimya Anabilim Dalı, 2011.

Sunum Planı

- Multiplex ölçümler
- Planar ve süspansiyon (BBM) ölçüm sistemleri
- Bead Based Multiplex (BBM) ölçüm prensibi
- Sistemin özellikleri, avantajları, dezavantajları
- Uygulama alanları
- Metabolik hastalıklar ile ilişkili çalışmalar

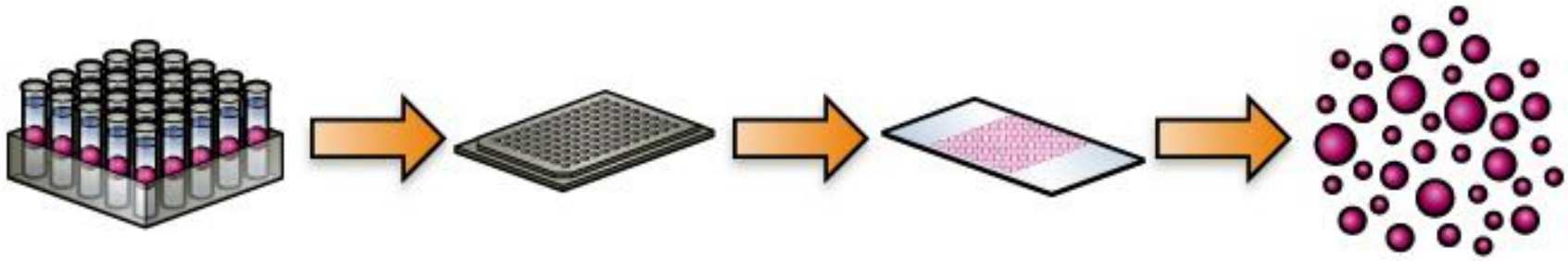
Multipleks assay nedir?

- Çoklu biyomarkırları ya da hücre içi sinyal modifikasyonlarını eş zamanlı olarak tespit etme yeteneği

Multiplex sistemler:
tek-analiz, çoklu-metabolit, çoklu hastalık

Uniplex sistemler:
tek-analiz, tek-metabolit, tek-hastalık

Multiplex ve paralel analizlerde endüstri ilerlemesi

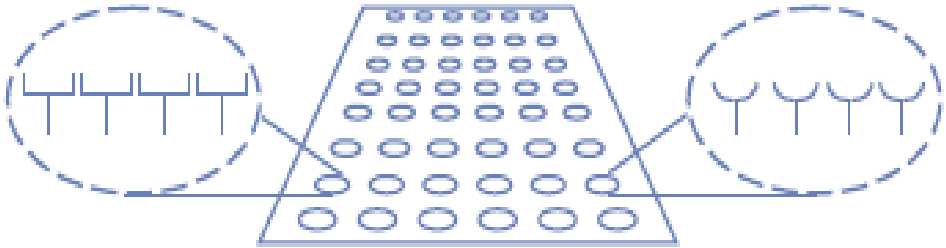


Test tüpleri

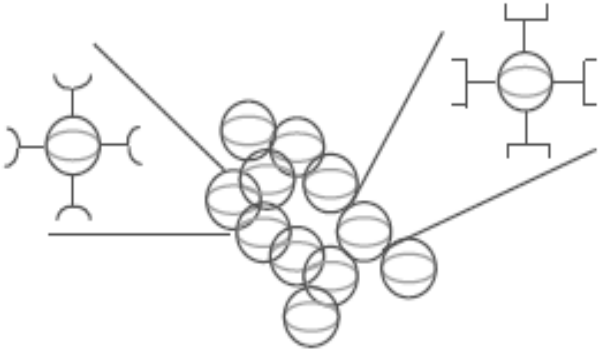
Mikrokuyucuklu levhalar

Mikroarray

Mikroboncuk tabanlı süspansiyon sistemler



Planar Array

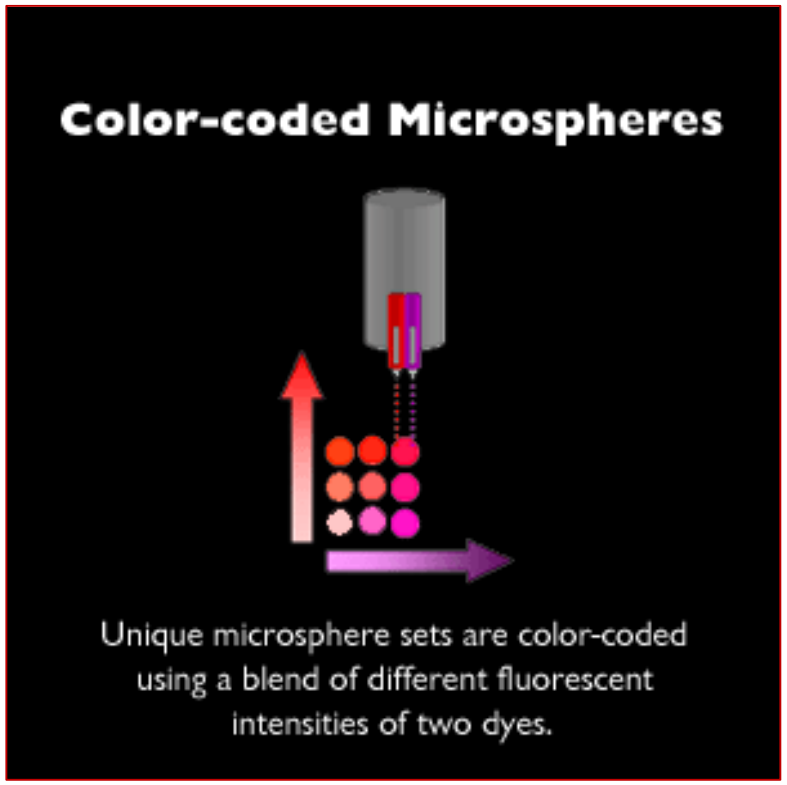


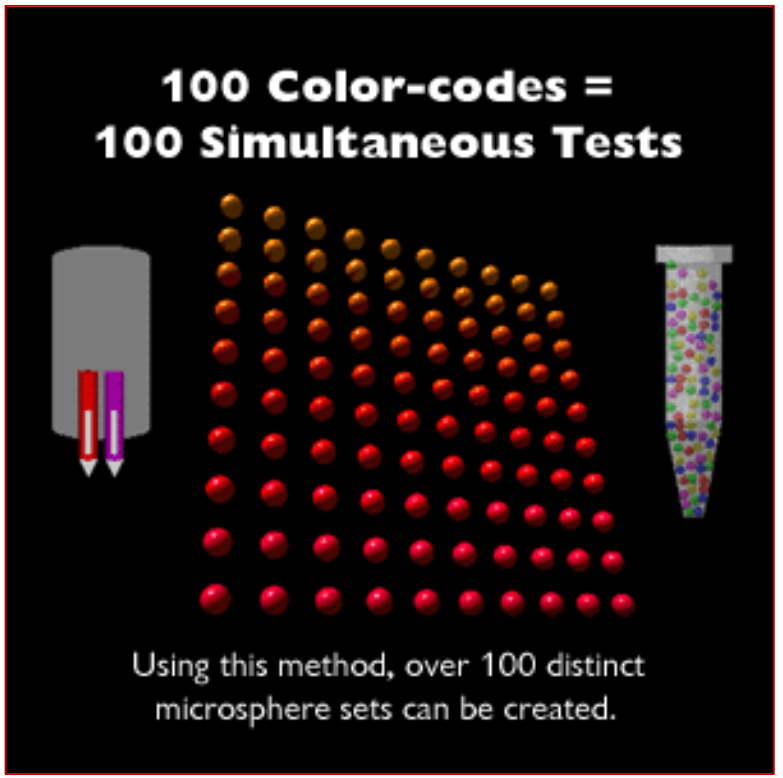
Süspansiyon array
(Bead-based array)

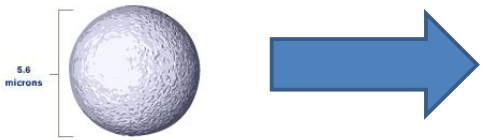
Platform	Multı-array/Multı-spot (Mesoscale Discovery)	Searchlight	Fastquant (Fast Quant System)
Capture Antibody Binding Surface	Carbon	Plastic	Nitrocellulose
Detection System	Electrochemiluminescent	Biotinylated Detector With Fluorescent Detection	Biotinylated Detector With Fluorescent Detection
Analytes/Plex	Up To 10	Up To 24	Up To 10
Image System	Ccd Camera Based	Ccd Camera Based	Ccd Camera Based
Customised Array	Yes	Yes	Yes
Kits For Designing And Building Your Own Assay	Yes	No	No
Reagent Company Commercial İnstrument	Waterman Scleicher& Schuell Bioscience	Aushon Biosystem	Waterman Scleicher& Schuell Bioscience

Platform	Luminex	Cytometric Bead Array (CBA)
Capture Antibody Binding Surface	Fluorescently Tagged Beads	Fluorescently Labeled Beads
Detection System	Biotinylated Detector With Fluorescent Detection	Phycoerythrin Conjugated Fluorescent Detectors
Analytes/Plex	Up To 100	Up To 100
Image System	Luminex Xmap Based System	Flow Cytometer With Dual Laser
Customised Array	Yes	Yes
Kits For Designing And Building Your Own Assay	Yes	Yes
Reagent Company Commercial İnstrument	Luminex (XMAP) And İts Partners	BD Becton,Dickinson And Others

BBM ölçüm prensibi

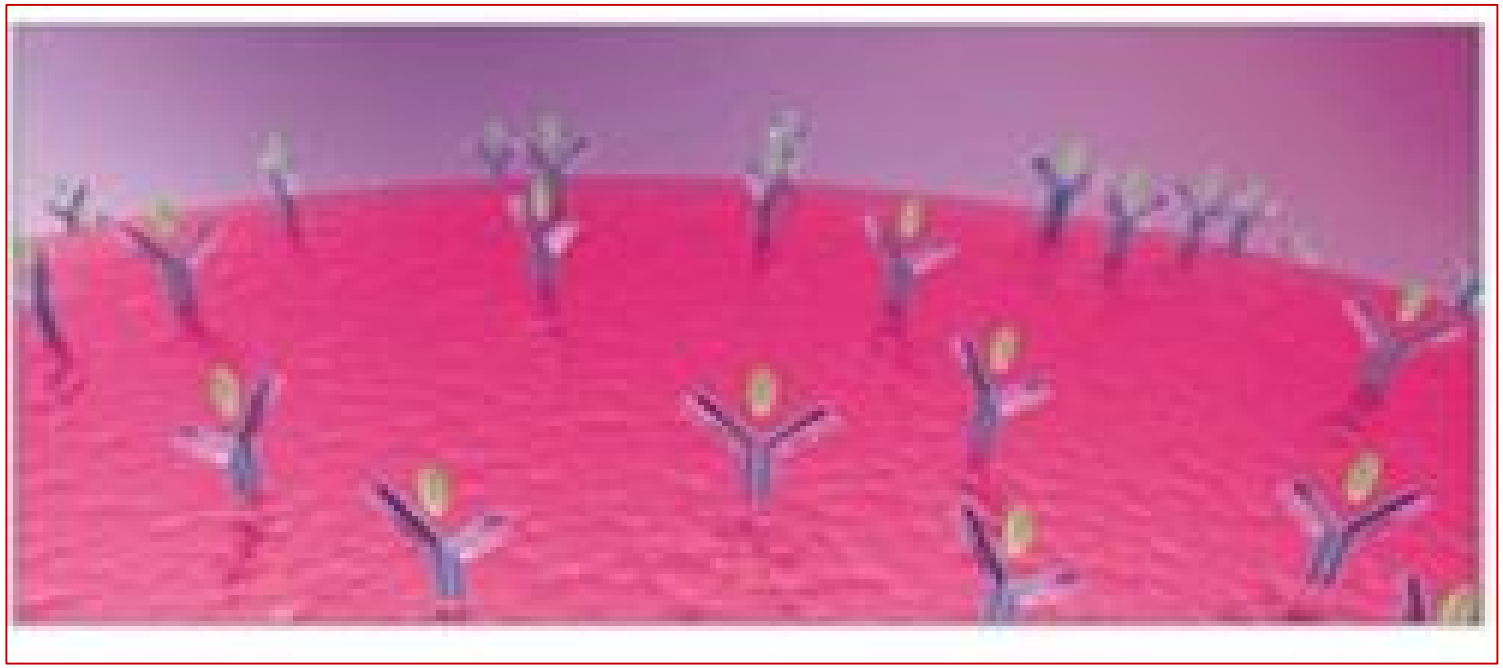






5.6 µm polystyrene bead

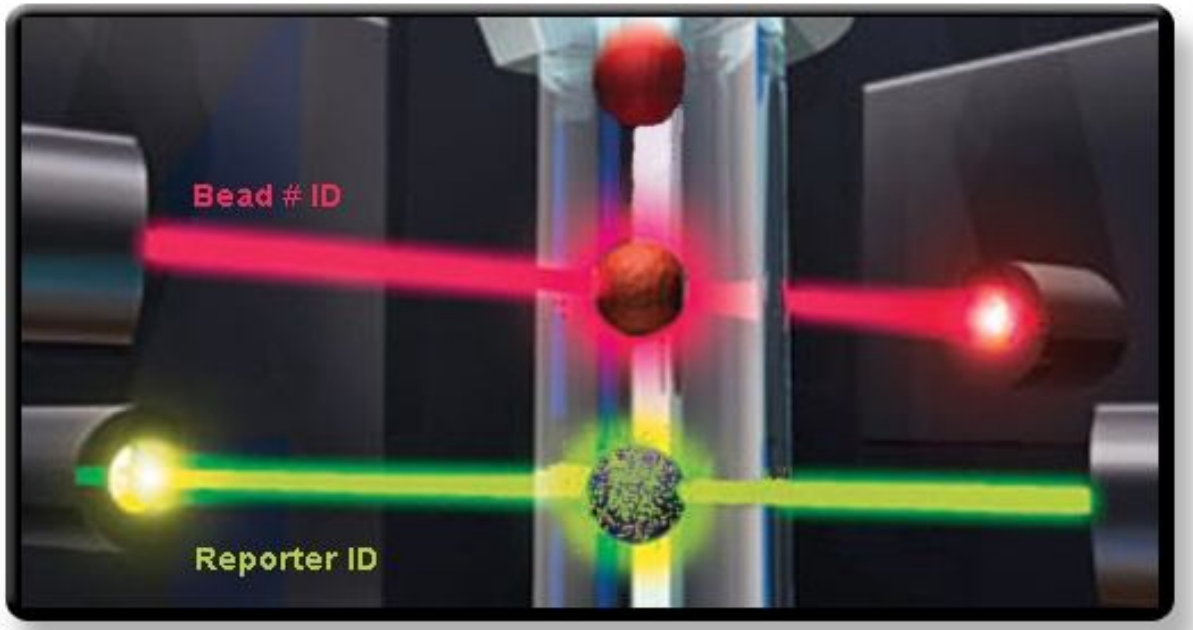
Mikrosferler yakalama antikoru ile kaplanır



Yakalama antikoru analiti bağlar

Floresan işaretli saptama antikoru eklenir

flow cell – herşeyin gerçekleştiği yer



Kırmızı lazer boncuğu – yani hedefi – okur.

635 nm Diod lazer

Yeşil lazer analit miktarını tespit eder.

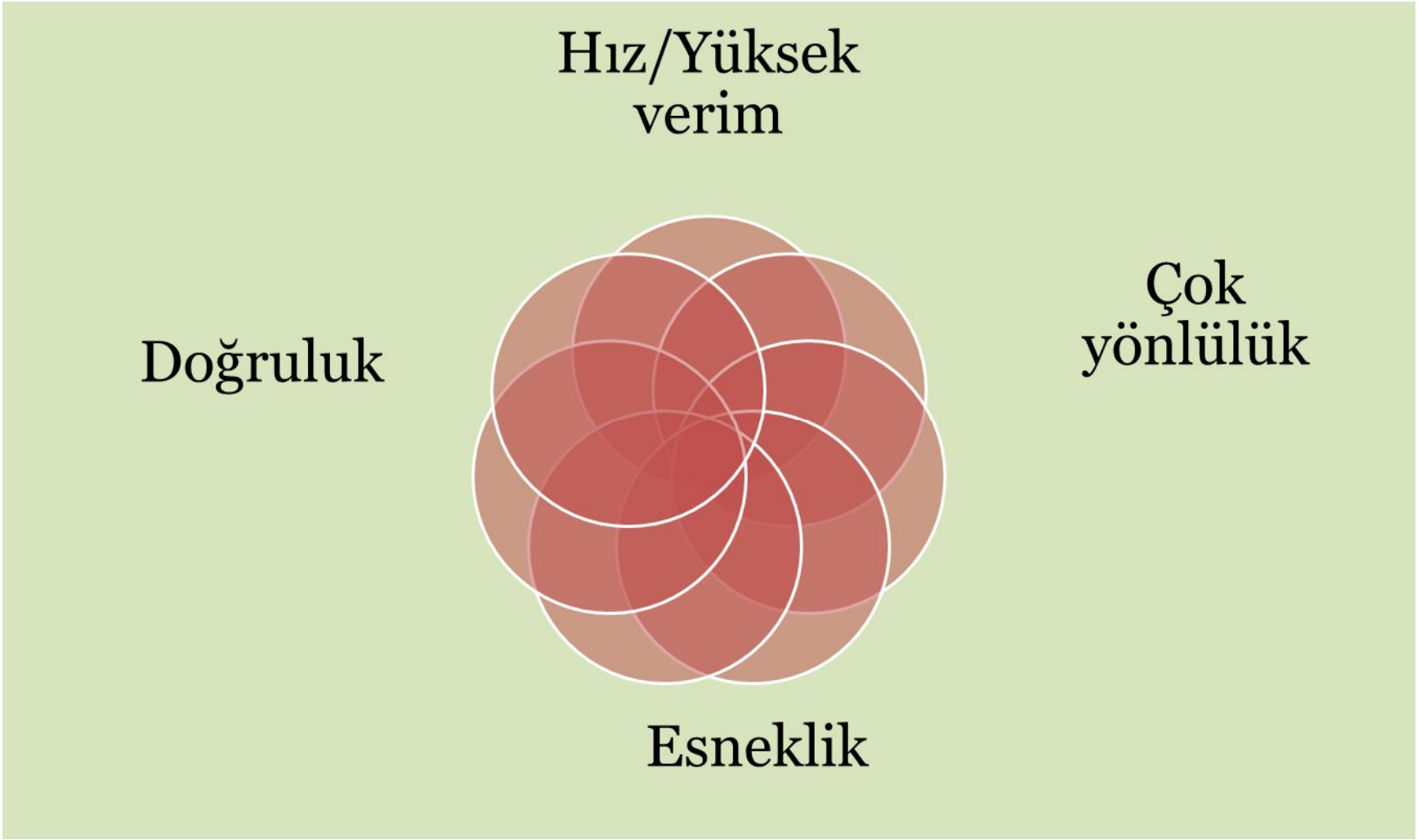
523 nm,Nd -Yag lazer

BBM ölçüm sistemlerinin özellikleri

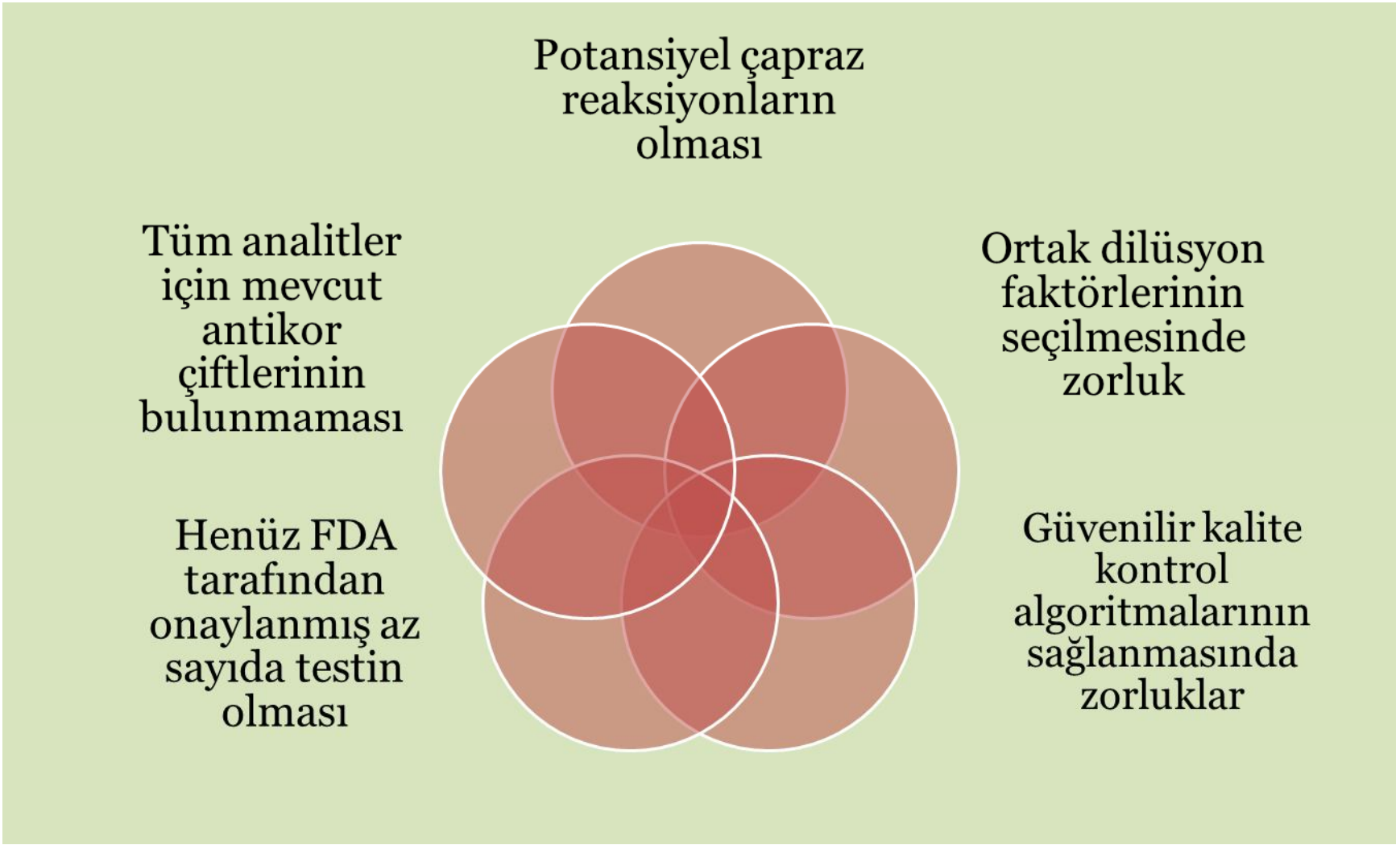
- Her kuyuda 100 ölçüm
- Düşük numune hacmi (25-50 µl)
- Günde 1000 örnek
- Yüksek tekrarlanabilirlik
- Sensitivite ng/ml
- 15 yıldan fazla geçmiş
- Yeni test geliştirme imkanı

- İş verimi yüksek
- Açık erişimli
- Kaynak çeşitliliği (Nükleik asit,Antijen, Antikor, Reseptör)
- Ölçümle eş zamanlı ölçümün kalite kontrolü

Sistemin avantajları



Sistemin dezavantajları



Sistem performansı neye bağlıdır?

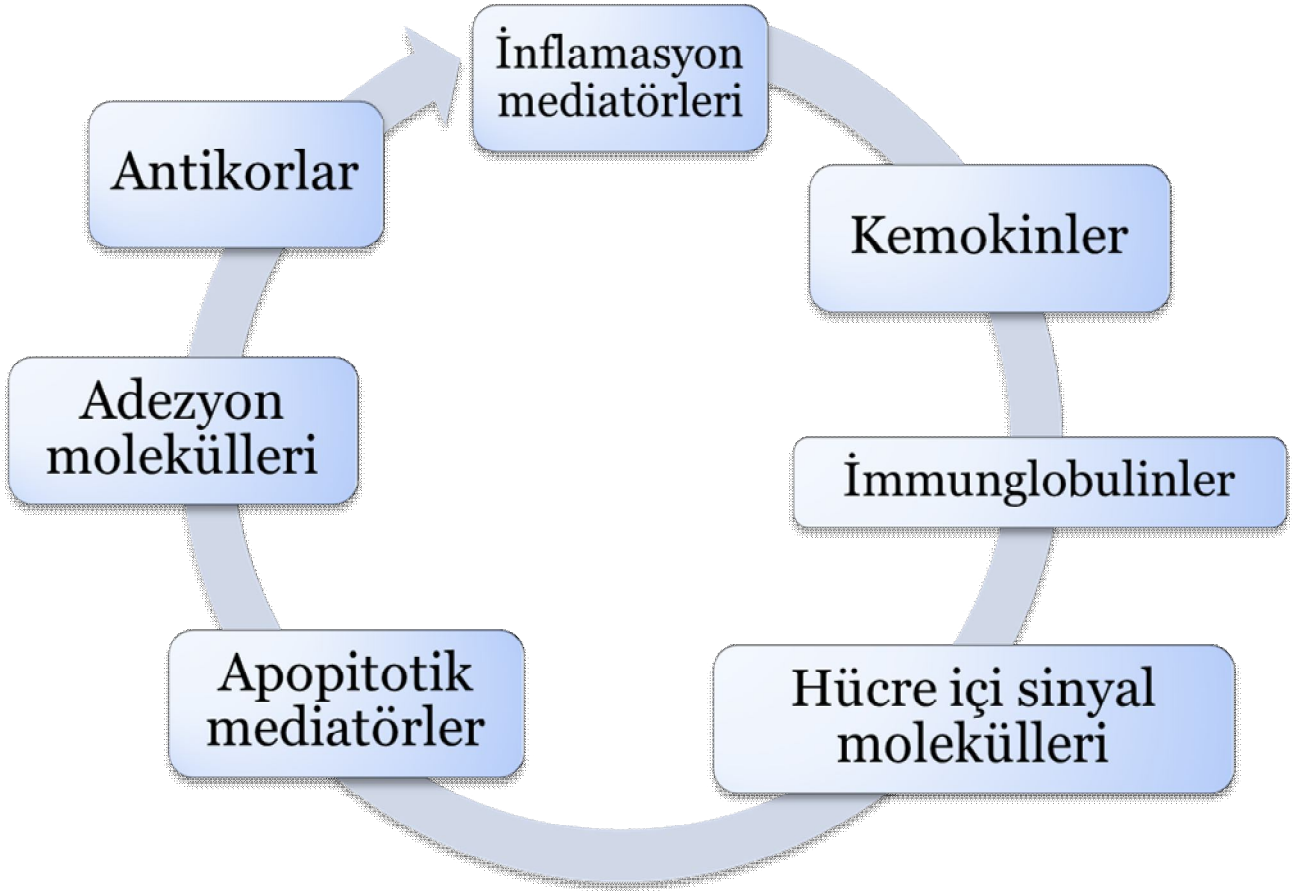
Bu yüzden test platformunun seçimi test sensitivitesine, analitin biyolojik konsantrasyona bağlıdır

Elisa'ya göre üstünlükleri

- Tek numunede 100 'e varan analit ölçümü
- Esnektir
- Daha küçük numune hacmi ister (25 µl)
- Daha hızlıdır.
- Karşılaştırılabilir duyarlılık

Numune ↓ **Zaman** ↓ **Maliyet** ↓ **Veri** ↑

BBM çeşitli analitlerin ölçülmesi için testlerin tasarlanması ve yapılmasına olanak sağlar.



Uygulamada mevcut kitler

APPLICATION	AVAILABLE KITS*	COMPANY
Allergy Testing	Alternaria,Bermuda Grass, Cat Dander, Egg White, Milk, Mite Pternoyssinus, Mountain Cedar, Short Ragweed, Timothy Grass,	ImTech
Autoimmune	beta-2 Microglobulin, Centromere B, Chromatin, DNA,ENA Profile 4 (SSA, SSB, Sm,RNP), ENA Profile 5 SSA, SSB, Sm, RNP, Scl-70), ENA Profile 6 (SSA, SSB, Sm, RNP, Scl-70, Jo-1), Gliadin A, Gliadin G , Histone, Histone H1, Histone H2A, Histone H2B, Histone H3, Histone H4, HSP-27 pS82, HSP-27 Total, HSP-32, HSP-65, HSP-71, HSP-90 a, HSP-90 b, Jo-1, PCNA PR3, PR3 (cANCA),RF, Ribosomal P RNP RNP-A, RNP-C, SCF Scl-70,Serum Amyloid P , SLE Profile 8 (SSA, SSB, Sm, RNP, Scl-70, Jo-1, Ribosome-P, chromatin) , Sm, Smith SSA SSB Streptolysin O TPO,TransglutaminaseA,Transglutaminase G	RBM

Cancer Markers	Alpha Fetoprotein , Cancer Antigen 125 , Carcinoembryonic Antigen , PSA, Free	RBM
Cardiac Markers	Creatine Kinase-MB , Endothelin-1 PAP , SGOT,TIMP-1	RBM
Cytokine	Abeta 40, Abeta 42, BDNF, DR-5, EGF ENA-78, Eotaxin, Fatty Acid Binding Protein, FGF-basic, G-CSF, GCP-2, GM-CSF, GRO alpha, GRO-KC, HGF I-TAC, ICAM-1, IFN-alpha, IFN-gamma, IL-10, IL-11, IL-12, IL-12, p40, IL-12, p40/p70, IL-12, p70, IL-13, IL-15, IL-16, IL-17, IL-18, IL-1alpha, IL-1beta, IL-1ra, IL-1ra/IL-1F3, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, JE/MCP-1,KC KC/GROa, LIF IL-8, IL-9,IP-10, JE/MCP-1, KC KC/GROa LIF MCP-3MCP-5	B-R Bios Linco

Endocrine	ACTH, Adiponectin, Amylin, C-Peptide, Calcitonin, CRF, FGF-9,FSH, GH, GLP-1, Glucagon, Insulin, Leptin, LH, Lipoprotein (a), PAI-1(active), PAI-1 (total), Prolactin, Resistin, T3, T4, TBG, Thyroglobulin, TSH	Linco RBM
Gene Expression	IL6R, ACTB, BAD, BAK1 (BAK), BCL2, BCL2L1 (BCL-XL), CDKN1A,(CDKN1), CFLAR, (CFLIP), , CSF2, GAPD, IFN-gamma, IL-1 beta, IL-10, IL-2, IL-6, IL-8, NFKB2, NFKBIA, (NFKIA), NKFB1, PPIB, Ptk2B (RAFTK), RELA, RELB, TNF, TNFAIP3 (A20), TNFRSF6 (FAS),TNFSF6 (FASL), VEGF	Bios MBio
MMP	MMP-1, MMP-12, MMP-13, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9	RD Bios

Genotyping	FlexMAP™, Mitochondrial DNA Screening, Tag-It™ Mutation Detection Kit, Y-SNP Identification	Bio Mira TmBio
Infectious Disease	Adenovirus, Bordetella pertussis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Cholera Toxin, Cholera Toxin b, Clostridium piliforme (Tyzzer's), Cytomegalovirus, Diphtheria Toxin, EDIM (Epidemic diarrhea of infant mice), Epstein-Barr EA, Epstein-Barr NA, Epstein-Barr VCA, HBV Core, HBV Envelope, HBV Surface (Ad), HBV Surface (Ay), HCV Core, HCV NS3, HCV NS4, HCV NS5, Helicobacter pylori, Hepatitis A, Hepatitis D, HEV orf2 3KD, HEV orf2 6KD, HEV orf3 3KD, HIV-1 gp120, HIV-1 gp41, HIV-1 p24, HPV, HSV-1 gD, HSV-1/2	RBM

Isotyping	IgA,IgE,IgG1,IgG2 alpha, IgG2beta, IgG3, IgM light chain	UP RBM
Metabolic Markers	Apolipoprotein A-1,Apolipoprotein A-II, Apolipoprotein B, Apolipoprotein C-II, Apolipoprotein C-III, Apolipoprotein E, beta-2 Glycoprotein, Collagen Type 1, Collagen Type 2, Collagen Type 4, Collagen Type 6, Glutathione S Transferas,Pancreatic Islet Cells, tTG (Celiac Disease)	RBM Linco
Tissue Typing	HLA Class I and II, HLA Class I Single Antigen Antibody, Group 1, HLA Class I Single Antigen Antibody, Group 2, PRA Class I, PRA Class I and II, PRA Class II, SSO Class I HLA-A, SSO Class I HLA-B, SSO Class I HLA-C, SSO Class II DP, SSO Class II DQB1, SSO Class II DRB1	Lambda

Kinase Phosphorylated Protein	Akt, Akt (Ser473), Akt (total), Akt/PKB (total), Akt/PKBpS473, ATF2 (Thr71), ATF2 (total), CREB (pS133), CREB (Total), Erk 1/2(pTpY185/187), Erk 1/2 (Total), Erk-2, Erk1 (Thr202/Tyr204), Erk1/2 (Thr202/Tyr204, Thr185/Tyr187), Erk2 (Thr185/Tyr187), Erk2 (total),GSK 3beta (pS9), GSK-3a/b (Ser21/Ser9), GSK-3beta, IGF 1R	UP Bios
Transcription Factors-Nuclear Receptors	AP-2, CREB, EGR, HIF-1, NF-1, NFAT, NFkB Gene Family, PPAR, SRE, YY1	Bios MBio

FlowCytomix multiplex kits

- **Human Adhesion 6plex**
  (sE-selectin, sICAM-1, sICAM-3, sP-selectin, sPECAM-1, sVCAM-1)
- **Human Cardiovascular 7plex**
  (sCD40L, IL-6, IL-8, MCP-1, sP-selectin, t-PA, sVCAM-1)
- **Human Chemokines 6plex**
  (IL-8, MCP-1, MIG, MIP-1a, MIP-1b, G-CSF)
- **Human Obesity 9plex**
  (sCD40L, sICAM-1, IL-6, Leptin, MCP-1, MPO, Osteoprotogerin, Resistin, sTNF-R)

Bio-plex platformu ile metabolik belirteçlerin gün-içi ve günler-arası %CV değerleri

Analit	Gün-içi %CV	Günler-arası %CV
Adiponektin	4	3
Ghrelin	5	4
GIP(gastrik inh polipeptit)	4	10
GLP-1	6	11
Glukagon	6	6
İnsülin	6	4
Leptin	4	3
PAI-1(plazminojen akt inh)	5	2
Resistin	4	4

FDA Onayı olan kitler

Endikasyon	Hedef	Satıcı firma	Platform
Allerji,çöliak	Antikor	INOVA Diagnostics	Luminex
Allerji,genel	Antikor	ImmuneTech	Luminex
Otoimmun	Antikor	Bioarray	Bead array
Otoimmun	Antikor	Biomedikal Diagnostics, Inova Diagnostics	Luminex
Otoimmun	Antikor	Zeus Scientifics, Bio-Rad Laboratories	Luminex
İnfeksiyon hastalıkları	Antikor	Bio-Rad Laboratories, Focus Diagnostics	Luminex

y4

Numune Tipleri

Serum
Plazma
İdrar
Ağız sıvısı
Gözyaşı
Bronko-alveolar sıvı
Kültür süpernatantı
Doku lizatı
Kan spotları
Burun yıkama sıvısı
Serebro spinal sıvı
Semen

Slayt 31

y4 yAYGIN OLARAK KLİNİKTE KULLANILAN NUMUNE TİPLERİ YANINDA GÖZYAŞI ,bos GİBİ AZ NUMUNE MİKTARI OLAN TESTLER İÇİN UYGULABİLİR OLMASI EN ÖNEMLİ AVANTAJLARINDANDIR.

yasemin; 17.11.2011

Eotaxin and Interleukin-4 Levels and Their Relation to Sperm Parameters in Infertile Men

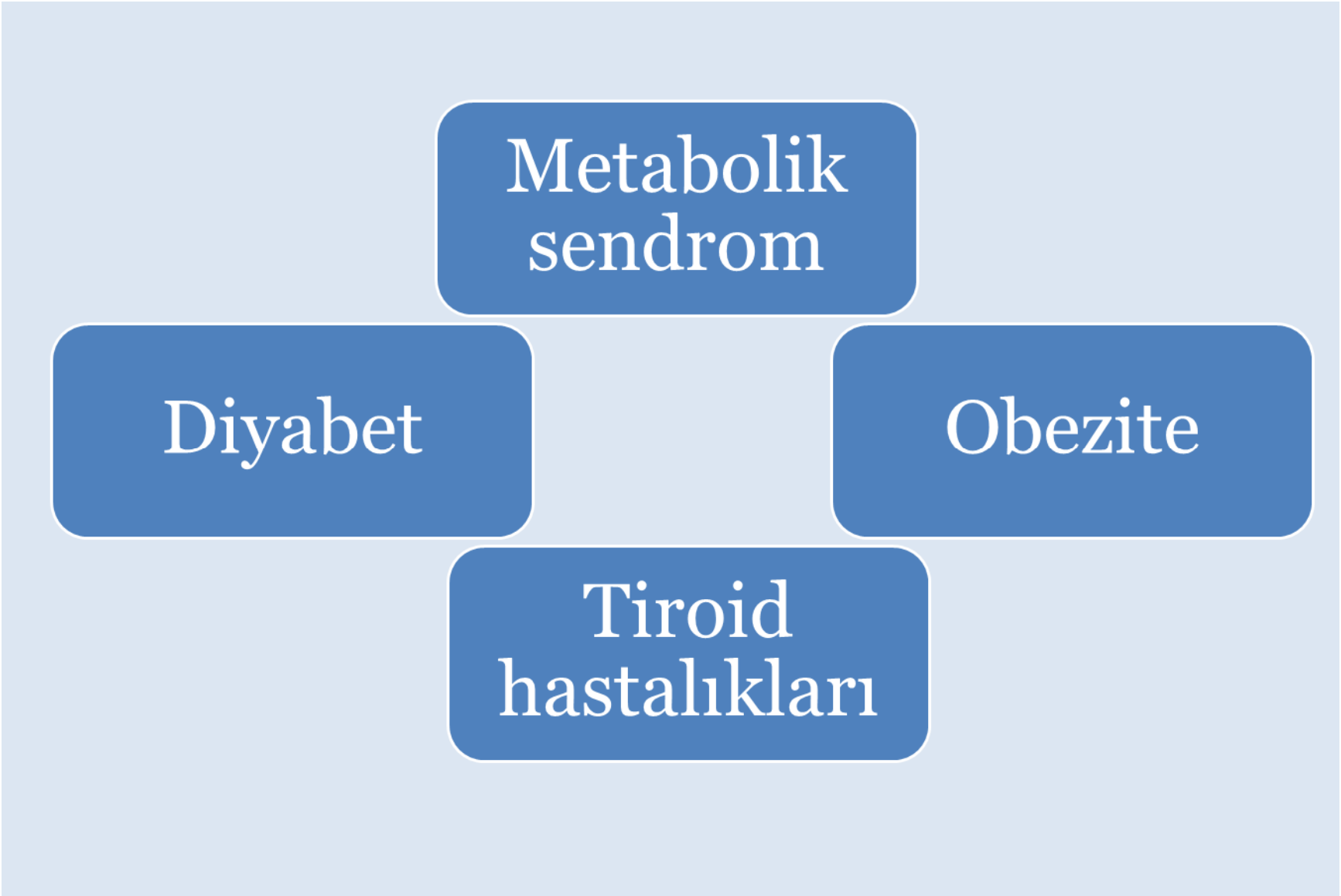
İnfertil Erkeklerde Eotaksin ve İnterlökin-4 Düzeyleri ve Sperm Parametreleri ile İlişkisi

Türkan YİĞİTBAŞI, MD,[a]
Yasemin BASKIN, MD,[b]
Gökhan AFACAN, MD,[c]
Füsun KARAARSLAN, MD,[d]
Cevat TAHERİ,[e]
Dilek ASLAN, MD[d]

ABSTRACT **Objective:** Male factor infertility accounts for 30% to 50% of the total infertile couples seeking for infertility treatment. In about 40-60% of these men, a specific etiology can not be found. The aim of this study was to confirm the presence of eotaxin and interleukin-4 (IL-4) in human seminal plasma, to show the differences between eotaxin and IL-4 concentrations in fertile and infertile men, and to show the potential relationship between eotaxin and IL-4 levels in semen and spermiogram parameters. In literature, this is the first study that evaluates eotaxin in the human seminal plasma. **Material and Methods:** The participant of the study was 55 infertile males with abnormal semen parameters as study group and 16 healthy volunteers with normal sperm parameters as the control group. Semen samples were classified according to criteria of the World Health Or-

Turkish Journal Of Medical Sciences, 2010

- Metabolik hastalıklar çok sebepli, çok genli hastalıklardır.
- Karmaşık hücresel işlevleri araştırmak için tek analit ölçümü yeterli olmamaktadır.
- Bu durum panellerle aşılmaya çalışılsa da eş zamanlı ölçüm yapılamamaktadır.
- Multiplex sistemler bu açıdan metabolik hastalıkların tanı ve izleminde gelecek vadeden sistemlerdir.


Metabolik sendrom
Diyabet
Obezite
Tiroid hastalıkları

Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2010; 35 (3) ; 177–182.

Araştırma Makalesi [Research Article]

Yayın tarihi 06 Eylül, 2010 © TurkJBiochem.com

[Published online 06 September, 2010]

Obez Hastalarda Büyüme Hormonu, Leptin, Amilin, Glukagon Benzeri Peptid-1 Seviyeleri ile İnsülin Direnci Arasındaki İlişki

[Relationship Between The Levels of Growth Hormone, Leptin, Amylin, Glucagon Like Peptide-1 and Insulin Resistance in Obese Patients]

Türkan Yiğitbaşı[1],
Yasemin Baskın[2],
Gökhan Afacan[3],
Ayşın Harmanda[1]

ÖZET

Amaç: Obezlerde, glukoz dengesinde rol alan büyüme hormonu, leptin, amilin, glukagon benzeri peptit -1 seviyeleri ile insülin direnci arasındaki ilişkiye açıklık getirmektir.

Gereç ve Yöntemler: Çalışmamızda, vücut kitle indeksi (VKI) ≤24.9-18.9≥ olan, 21-72 (45.6±14.5) yaş aralığında 32 erişkin (26 kadın ve 6 erkek) kontrol grubu olarak; VKI≥24.9 olan 21-72 (52.4±10.78) yaş aralığında 68 erişkin obez (35 kadın ve 33 erkek)

Clinical Chemistry 51:7
1102–1109 (2005)

Molecular Diagnostics and Genetics

Multiplexed Analysis of Biomarkers Related to Obesity and the Metabolic Syndrome in Human Plasma, Using the Luminex-100 System

Mine Y. Liu,[1] Antonios M. Xydakis,[2] Ron C. Hoogeveen,[1] Peter H. Jones,[1] E. O'Brian Smith,[3] Kathleen W. Nelson,[4] and Christie M. Ballantyne[1*]

Background: The complex pathology of disease has sparked the development of novel protein expression profiling techniques that require validation in clinical settings. This study focuses on multiplexed analyses of adipocytokines and biomarkers linked to the metabolic syndrome, diabetes, and cardiovascular disease.

Methods: Multiplexed immunoassays using fluorescent microspheres and the Luminex-100 system were performed on plasma from 80 obese patients (40 with the metabolic syndrome) before and after 6–8 weeks of diet-induced weight loss. Leptin, insulin, C-peptide, monocyte chemoattractant protein-1 (MCP-1), eotaxin, interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and IL-6 concentrations measured with multiplex panels from 3 different manufacturers were compared with results from commercial ELISAs. Detection limits and TNF-α, IL-8, and IL-6 (Linco, Biosource, Upstate, and R&D) with correlation coefficients of −0.107 to 0.318. Within- and between-run imprecision values for the multiplex method were generally <15%. Relative changes in plasma leptin and insulin concentrations after diet-induced weight loss were similar whether assessed by multiplex assay or ELISA.

Conclusion: Although this technology appears useful in clinical research studies, low assay sensitivity and poor correlations with conventional ELISA methods for some analytes with very low plasma concentrations should be considered when using the Luminex platform in clinical studies.

Obesity has reached epidemic proportions in the United States; ~30% of US adults are obese, and the percentage is

Eur J Endocrinol. 2007 Mar;156(3):387-94.

Lower plasma adiponectin is a marker of increased intima-media thickness associated with type 2 diabetes mellitus and with male gender.

Dullaart RP, de Vries R, van Tol A, Sluiter WJ.

Department of Endocrinology, University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands. r.p.f.dullaart@int.umcg.nl

Abstract

OBJECTIVE: We tested the extent to which altered plasma adipokine levels may contribute to the increased carotid artery intima-media thickness (IMT) associated with type 2 diabetes mellitus and with male gender, independently of conventional cardiovascular risk factors, insulin resistance, and plasma C-reactive protein (CRP).

DESIGN: IMT (mean of three segments of both carotid arteries by ultrasonography), insulin resistance (homeostasis model assessment; HOMA(ir)), plasma CRP, lipids, adiponectin, leptin, resistin, and tumor necrosis factor-alpha (TNF-alpha) were measured in 84 type 2 diabetic patients and 85 control subjects.

RESULTS: In diabetic patients, IMT (P<0.001), mean arterial pressure (P<0.001), HOMA(ir) (P<0.001), plasma CRP (P=0.003), triglycerides (P=0.037), leptin (P=0.023), resistin (P=0.003), and TNF-alpha (P=0.003) levels were higher, whereas high-density lipoproteins (HDL) cholesterol (P<0.001) and adiponectin (P<0.001) levels were lower compared with control subjects. Plasma adiponectin (P<0.001) and leptin (P<0.001) were substantially lower in men than in women. IMT was positively and independently associated with age (P<0.001), diabetes (P=0.049), and male gender (P=0.002) in a multivariate regression model, not including other variables. Further analyses showed that IMT was positively related to age (P<0.001) and plasma triglycerides (P=0.038) and negatively to adiponectin (P<0.001), without independent effects of diabetes, gender, and HOMA(ir).

CONCLUSIONS: Increased IMT in type 2 diabetes may in part be explained by lower plasma adiponectin and higher triglycerides, but not by leptin, resistin, and TNF-alpha. The gender effect on IMT is related to lower plasma adiponectin.

Clin Chem. 2010 Aug;56(8):1320-8. Epub 2010 Jun 8.

A multiplex immunoassay for human adipokine profiling.

Schipper HS, de Jager W, van Dijk ME, Meerding J, Zelissen PM, Adan RA, Prakken BJ, Kalkhoven E.

Department of Metabolic and Endocrine Diseases, UMC Utrecht, the Netherlands.

Abstract

BACKGROUND: Adipose tissue secretory proteins, called adipokines, play pivotal roles in the pathophysiology of obesity and its associated disorders such as metabolic syndrome, type 2 diabetes, and cardiovascular disease. Because methods for comprehensive adipokine profiling in patient plasma and other biological samples are currently limited, we developed a multiplex immunoassay for rapid and high-throughput measurement of 25 adipokines in only 50 microL of sample.

METHODS: (Pre)adipocyte and ex vivo cultured adipose tissue supernatants were generated and together with plasma from 5 morbidly obese patients and 5 healthy and normal weight controls used to develop the adipokine multiplex immunoassay and test its usefulness in biological samples. We assessed adipokine dynamic ranges, lower limits of detection and quantification, cross-reactivity, intra- and interassay variation, and correlation with adipokine ELISAs.

RESULTS: The limits of quantification and broad dynamic ranges enabled measurement of all 25 adipokines in supernatants and patient plasmas, with the exception of TNF-alpha in plasma samples. Intraassay variation was <10% for all adipokines; interassay variation was < 15%. The multiplex immunoassay results correlated significantly with ELISA measurements. Plasma adipokine profiling showed significantly higher concentrations of the novel adipokines cathepsin S (5.1 x 10(4) vs 4.3 x 10(4) ng/L, P = 0.003) and chemerin (4.1 x 10(5) vs 2.7 x 10(5) ng/L, P = 0.0008) in morbidly obese patients than normal weight controls, besides the established differences in adiponectin and leptin concentrations.

CONCLUSIONS: Our findings underscore the relevance of the novel adipokines cathepsin S and chemerin, but foremost the potential of this novel method for both comprehensive adipokine profiling in large patient cohorts and for biological discovery.

Mol Vis. 2008 Mar 27;14:637-43.

Multiplex bead analysis of vitreous and serum concentrations of inflammatory and proangiogenic factors in diabetic patients.

Maier R, Weger M, Haller-Schober EM, El-Shabrawi Y, Wedrich A, Theisl A, Aigner R, Barth A, Haas A.

Department of Ophthalmology, Medical University of Graz, Graz, Austria. richardmaier@chello.at

Abstract

PURPOSE: To investigate the role of inflammatory and angiogenic factors in the pathogenesis of diabetic retinopathy, we determined, in diabetic patients and controls, vitreous and serum concentrations of interferon-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, regulated upon activation, normal T-expressed and secreted (RANTES), and vascular endothelial growth factor (VEGF).

METHODS: We recruited 36 probands with type 2 diabetes mellitus (15 noninsulin-dependent and 21 insulin-dependent) and 69 normal controls. Using Cytometric Bead Array Technology, we measured vitreous and serum concentrations of IP-10, MCP-1, MIP-1alpha, MIP-1beta, RANTES, and VEGF.

RESULTS: In diabetic patients the mean vitreous levels of IP-10, MCP-1 and VEGF were significantly higher compared normal controls. [IP-10 (pg/mL) 254.84 +/-311.67 versus 78.90 +/- 67.94 ($p<0.001$); MCP-1 (pg/mL) 1127.14 +/- 738.91 versus 700.80 +/-419.21 ($p=0.002$); VEGF (pg/mL) 954.98 +/- 2315.09 versus 37.90 +/- 28.51($p<0.001$)]. Vitreous levels of VEGF correlated with vitreous levels of both IP-10 and MCP-1 ($p<0.05$). MIP-1beta, RANTES, and VEGF mean serum levels were significantly raised in diabetic probands while IP-10, MCP-1, and MIP-1alpha serum levels showed no significant elevation compared to controls [IP-10 (pg/mL) 346.20 +/- 287.36 versus 328.74 +/-352.35 ($p=0.88$); MCP-1(pg/mL) 133.10 +/- 89.10 versus 141.47 +/- 222.15 ($p=0.50$); MIP-1beta (pg/mL) 184.40 +/- 100.20 versus 139.56 +/- 151.38 ($p=0.003$); RANTES (pg/mL) 51336.23 +/- 19940.31 versus 33629.2 +/- 33301.0 ($p=0.002$); VEGF (pg/mL) 304.88 +/- 257.52 versus 154.45 +/- 114.78 ($p<0.001$)].

CONCLUSIONS: Our results suggest that in diabetics, there is an upregulation of IP-10, MCP-1, and VEGF in the vitreous and an upregulation of MIP-1beta, RANTES, and VEGF in the serum. These findings support the concept of an angiogenic and inflammatory element in the development of diabetic retinopathy.

Eur J Endocrinol. 2008 Feb;158(2):179-87.

Weight loss larger than 10% is needed for general improvement of levels of circulating adiponectin and markers of inflammation in obese subjects: a 3-year weight loss study.

Madsen EL, Rissanen A, Bruun JM, Skogstrand K, Tonstad S, Hougaard DM, Richelsen B.

Department of Endocrinology and Metabolism C, Aarhus University Hospital, Aarhus Sygehus, Tage Hansensgade 2, DK-8000 Aarhus C, Denmark.

Abstract

OBJECTIVE: To investigate the effects of: I) short- (8 weeks), II) long-term (3 years) weight loss, and III) the degree of weight loss on circulating levels of adiponectin, high sensitive-C reactive protein (hs-CRP), and fibrinogen in obese subjects. Moreover, to evaluate the effect of the lipase inhibitor, orlistat, on these parameters.

DESIGN: Weight loss induced in 93 obese subjects (mean weight: 108.9+/-15.8 kg) through 8-week very-low-energy diet (VLED, 800 kcal/day) followed by randomization to orlistat or placebo together with lifestyle intervention for further 3 years. Adiponectin and hs-CRP were measured at baseline, after 8 weeks of VLED and 6, 12, and 36 months after the VLED by flowmetric xMAP technology (Luminex Multi-Analyte Profiling System, Luminex Corp., Austin, TX, USA). Fibrinogen was measured in a coagulation assay.

RESULTS: Weight loss after VLED treatment was 14.3+/-4.5 kg and after 3 years 7.7+/-8.7 kg. Orlistat-treated subjects regained 3.9 kg less than placebo-treated from the end of the VLED to 3 years (P=0.01). No differences were detected between the two groups regarding changes in adiponectin, hs-CRP, or fibrinogen. Accordingly, the groups were combined for further analyses. Serum adiponectin increased by 22% (P<0.05) after the VLED but returned to baseline after 3 years. Both short- and long-term weight losses needed to be in excess of 10% (approximately 12 kg) in order to increase adiponectin levels significantly. Weight loss was associated with a significant decrease in hs-CRP. Fibrinogen decreased by 12% (P<0.05) after 2 years

Physiol. Res. 59: 79-88, 2010

The Influence of Obesity and Different Fat Depots on Adipose Tissue Gene Expression and Protein Levels of Cell Adhesion Molecules

L. BOŠANSKÁ[1], D. MICHALSKÝ[2], Z. LACINOVÁ[1], I. DOSTÁLOVÁ[1], M. BÁRTLOVÁ[1], D. HALUZÍKOVÁ[1,3], M. MATOULEK[1], M. KASALICKÝ[4], M. HALUZÍK[1]

Visceral adipoz dokuda VCAM-1,ICAM-1,E-Selectin yüksel bulunmuştur.

Clin Biochem. 2005 Nov;38(11):966-72. Epub 2005 Oct 5.

Clinical evaluation of a microsphere bead-based flow cytometry assay for the simultaneous determination of anti-thyroid peroxidase and anti-thyroglobulin antibodies.

González C, García-Berrocal B, Talaván T, Casas ML, Navajo JA, González-Buitrago JM.

Servicio de Bioquímica, Laboratorio de Autoinmunidad, Hospital Universitario, Salamanca, Spain.

Abstract

OBJECTIVES: Multiplexing technologies based on the use of microspheres as the solid phase have opened new possibilities for the analysis of autoantibodies. As an alternative to the traditional immunoassays, it is possible to use these methods in combination with flow cytometry for simultaneous measurement of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies.

DESIGN AND METHODS: We studied 127 serum samples sent to our laboratory for the quantitation of anti-TPO and anti-Tg antibodies. Clinical information was available for all of the patients studied. The samples were analyzed simultaneously for both antibodies by flow cytometry (FIDIS, BMD, France), and individually for each of the antibodies by an automated enzyme immunoassay (UniCap, Pharmacia Diagnostics, Germany).

RESULTS: A significant association between the results was observed. The kappa agreement indices between the methods were 0.859 and 0.832 for anti-TPO and anti-Tg, respectively. Discrepant results between the two techniques were observed with no common cause. Anti-TPO and anti-Tg antibodies exhibited a non-Gaussian distribution. The areas under the ROC curves were similar for both methods used; for anti-TPO, 0.884 (Pharmacia) and 0.853 (BMD), and for anti-Tg, 0.833 (Pharmacia) and 0.837 (BMD).

CONCLUSION: Cytometry multiplex technology offers a true alternative to conventional immunoassays in the analysis of anti-TPO and anti-Tg antibodies.

Neden bu kadar çok Elisa ve BBM karşılaştırma çalışması yapılmış?

Clin Chem. 2000 Sep;46(9):1422-4.

Simultaneous measurement of thyroxine and thyrotropin from newborn dried blood-spot specimens using a multiplexed fluorescent microsphere immunoassay.

Bellisario R, Colinas RJ, Pass KA.

Division of Genetic Disorders, Wadsworth Center, New York State Department of Health, Albany, NY 12201-0509, USA. bellisar@wadsworth.org

Sonuç olarak,

- Bu testlerin klinik kullanıma girmesi metabolik hastalıkların tanı ve izlemine katkı sağlayacaktır.
- Ancak sınırlı sayıda multiplex test FDA onayı almıştır.
- Gelecek vadeden teknolojilerdir.

REFERANSLAR:

- 1-Multiplexed Analysis Of Biomarkers Related To Obesity And The Metabolic Syndrome İn Human Plasma, Using The Luminex-100 System. Liu M.Y, Xydakis A.M, Hoogeveen R.C, Jones P.H, O'brian Smith E, Nelson K.W, Ballantyn C.M. Clinical Chemistry.2005;51(7):1102–1109 .
- 2-Multiplex Assays For Biomarker Research And Clinical Application: Translational Science Coming Of Age. Fu Q, Schoenhoff F.S, Savage W.J, Zhang P, Van Eyk J.E.Proteomics Clin. Appl. 2010 ;4:271–284.
- 3- Comparison Of Multiplex İmmunoassay Platforms.Fu Q,Zhu J,Van Eyk Je.Clin Chem 2010;56(2):314-8.
- 4- Measurement And Quality Control Issues İn Multiplex Protein Assays: A Case Study. Ellington A.A, Kullo I.J, Bailey K.R, Klee G.G. Clin Chem. 2009; 55(6): 1092–1099.
- 5- Multi-analyte İmmunoassay. Ekins Rp.Journal Of Pharmaceutical And Biomedical Analysis.1989; 7(2):155-168.
- 6- Us Food And Drug Administration. Guidance For İndustry And Fda Staff: Pharmacogenetic Tests And Genetic Tests For Heritable Markers. (Accessed October 2011].
- 7- Multiplexed Protein Measurement: Technologies And Applications Of Protein And Antibody Arrays. Kingsmore S.F. Nat Rev Drug Discov. 2006; 5(4): 310–3208.
- 8- Antibody-based Protein Multiplex Platforms: Technical And Operational Challenges. Ellington A.A, Kullo Ij, Bailey K.R, Klee G.G. Clin Chem. 2010; 56(2): 186–193.
- 10- Rapid Detection Of Antibodıes In Sera Using Multiplexed Self-assembling Bead Arrays. Wonga J, Sibanib S, Lokkoa N.N, Labaerb J, Andersona K. J Immunol Methods.2009; 31: 171–182.
- 11-Multiplex Bead Array Assays: Performance Evaluation And Comparison Of Sensitivity To Elısa. Elshal M.F, Mccoy J.P. Methods. 2006; 38(4): 317–323.

Teşekkür ederim...