Novel Technology for Automated and High Throughput Multi-Target Detection of Cytokine and other Soluble Biomarkers

Bio-Plex System and Pro™ Magnetic Assays

2009
• Why and How to do Multiplexing?

• What are Bio-Plex Cytokines Assays?

• How to use Bio-Plex Assays for Profiling?

• Analysing Complex Samples Using Magnetic Assays

• Biomarker Detection in Clinical Samples
Bio-Rad Laboratories
life science research & diagnostics

Supplying Innovations for than 50 years
- Chromatography Solutions
- Electrophoresis Technology
- Proteomics and Imaging Systems
- PCR Technology
- Multiplex Protein Assays
- Protein Interaction Technology
Who is Christian?

• **Education:** Proteomics Expert, Specializing in Multiplex Assays
  – Engineering degree in Biotechnology
  – PhD in Molecular Biology
• **Experience:** 20+ years in working in labs, doing research

• **Company Areas:**
  • Bio-Rad and Protein Function Division
  • Protein Separation Techniques
  • Imaging Analysis and Proteomic System
  • Multiplex Immunoassays System
  • Protein Interaction Technologies

• **Functions:**
  • Provide Field Application Support
  • Introducing New Technologies
  • Ensure Customer Feedback & Satisfaction

• **Business ‘Passion’:**
  – support customers to explore new application areas
Analysis of Protein Networks

- Cell Physiology
- Cell-to-Cell Interaction
- Influence of External Factors

Chemokines – Where to go?

Cytokine – What to do?
**Immuno Assays**

**“Sandwich”-Immuno-Assay**

- Incubation with plasma or standard
- Matrix with capture Ab
- Detection with labelled secondary Ab

- e.g., cytokines

**Competitive-Immuno-Assay**

- Incubation with labelled standard & plasma
- Detection of labelled standard

- e.g., immunoglobulines
Multiplex Immuno Assay

RIA

ELISA

MIA

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Technology platform basics

Industry advances in parallel and multiplex analysis

- Test tubes
- Microwell plate
- Microarray
- Bead Technology: Microsphere-based suspension array
Protein Analysis single vs multiplex

Protein microarrays and proteomics
What is a Bio-Plex Bead Assay?

• In short, it is an Immuno Assay on a bead.

• Bio-Plex assays using a set of 5 micron beads instead of a microplate well.

• The beads have different colors and each color is associated with a specific analyte.

• Using beads enables us to run multiple analytes in a single well.
Multiplexing with Colored Bead Sets

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Multiplexing with Colored Bead Sets
Cytokine Assay Workflow

1. Mix antibody coated beads with samples, standards and controls

2. Add specific biotinylated detection antibodies

3. Detection: streptavidin-PE
   - 30 mins incubation
   - Wash between each step
   - 10 mins incubation

4. Read assays in Bio-Plex 200

Under 3 hours start to finish
Fluorescence Tags used for Detection

- **Phycobiliproteins**
  - *e.g.* **R-Phycoerythrin** (R-PE)
    - Intense long-wavelength excitation and emission to provide fluorescence that is relatively free of interference from other biological materials
    - Relatively large Stokes shifts with extremely high emission quantum yields
    - Very high water solubility
    - Homogeneous structure with defined molecular weights
Cytokine Assay Bibliography

Hundreds of Citations

• Human Samples
  – Blister Fluid
  – Bronchoalveolar Lavage Fluid
  – Cerebrospinal Fluid
  – Fat (Adipose) Interstitial Fluid
  – Nasal Lavage Fluid
  – Peritoneal Fluid
  – Plasma*
  – Serum*
  – Synovial Fluid
  – Tissue Culture Supernatant*

• Mouse Samples
  – Plasma*
  – Serum*
  – Bronchoalveolar Lavage Fluid
  – Synovial Fluid/Patellar Washouts
  – Tissue-Colon, Kidney, Lung, Nervous System & Spleen
  – Tissue Culture Supernatant*

• Rat Samples
  – Bronchoalveolar Lavage Fluid
  – Plasma*
  – Serum*
  – Tissue –Colon & Nervous System
  – Tissue Culture Supernatant*

* Validated by Bio-Rad
Industrial Bio-Plex USE

- Abbott
- Amgen
- AstraZeneca
- Bayer
- Biogen Idec
- Bristol-Myers Squibb
- Centocor (J&J)
- Charles River Labs (CRL)
- Convance
- Critical Therapeutics
- Eli Lilly
- Enzon Pharmaceuticals
- Genetech
- Genzyme
- GlaxoSmithKline (GSK)
- Institute for Bioanalytics (IBA)
- IBT Reference Labs
- Jackson Laboratories
- Merck
- MPI Research
- Nycomed
- Proctor & Gamble
- Pfizer
- Quest Pharmaceutical Services
- R.J. Reynolds
- Roche
- Sanofi-Aventis
- Schering-Plough
- Synta Pharmaceuticals
- Therakos (J&J)
- TGA Sciences
- Wyeth
Multiplex Cytokine Applications

- Cytokine Expression Profiling
  - Immunological Profiling of Mononucleocytes in Response to Mitogenic Stimulation
  - Active caspase-1 is a regulator of unconventional protein secretion
  - Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge
  - Define cytokine profile of highly pathogenic avian H5N1 influenza infection

- Biomarker Validation & Monitoring
  - Towards Discovery-driven Translational Research in Breast Cancer
  - Multiplex analysis of serum biomarkers in ovarian cancer patients
In Vivo Model for Drug Treatment to study of mode of action anti-inflammatory drugs
Protein Expression Profiles of 30 Analytes in PBMCs after Stimulation with Bacterial Toxins
17-plex Dose-Response Curves

Human Cytokine Panel

- IL-2
- IL-4
- IL-6
- IL-8
- IL-10
- GM-CSF
- IFN-γ
- TNF-α
- IL-1β
- IL-5
- IL-7
- IL-12 (p70)
- IL-13
- IL-17
- G-CSF
- MCP-1
- MIP-1β
ELISAs Weighing You Down? Try Bio-Plex.

The new automated Bio-Plex Pro™ research tools make Bio-Plex assays a more productive alternative.

In a single well, evaluate up to 50 cytokines, chemokines, or growth factors, and use focused diseases related assay panels to better understand:
- Acute phase response
- Cytokines
- Chemokines
- Inflammation
- Signal Transduction

Run fewer experiments, use less samples, and increase productivity with Bio-Plex multiplex assays.

For more information, go to www.bio-rad.com/bio-plex/

To find your local office, visit www.bio-rad.com/office_locator

Visit us on the Web at www.bio-rad.com

BIO-RAD

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Why Multiplex Analysis?

• Less Sample!
  – Scarce/precious samples

• Dramatically increase of information per single sample!
  – Maximize information obtained

• Reduce assay reagent volume, expense and labor time!
  – Save time

• Better interrelationships between related analytes!
  – Link molecules involved in different signaling pathways
Active Caspase-1 Is a Regulator of Unconventional Protein Secretion

Martin Keller,1 Andreas Rüegg,1 Sabine Werner,1 and Hans-Dietmar Beer1,*
1Institute of Cell Biology, Department of Biology, ETH Zurich, CH-8093 Zurich, Switzerland
*Correspondence: dietmar.beer@cell.biol.ethz.ch
DOI 10.1016/j.cell.2007.12.040
• siRNA-based method for analyzing caspase-1 dependent release of various cytokines in UV-irradiated human keratinocytes

• to determine whether caspase-1 specifically targets unconventional protein secretion or secretion in general

• After siRNA transfection cells were UV treated and the supernatant collected

• The remaining cells were lysed and stored at -70 C until the cytokine were analyzed with the 27-plex
Secreted Cytokines 4 hours after UVB irradiation of keratinocytes

Fig. 1. UVB-induced cytokine secretion from keratinocytes. Cytokines were measured in cell culture supernatants from keratinocytes 4 hr after UVB irradiation or in control cells using the Bio-Plex multiplex array system and human cytokine 27-plex panel. Bars represent values from single measurements. The 21 cytokines found to be secreted are shown.
Caspase-1 dependent cytokine expression and secretion of keratinocytes after UVB irradiation and siRNA inhibition of caspase-1

**Fig. 3.** Caspase-1-dependent cytokine expression and secretion of keratinocytes after UVB irradiation. Cytokines were measured in cell culture supernatants and cell lysates 4 hr after irradiation of keratinocytes transfected with siRNA specific for caspase-1 (●) or VEGF (■) using the Bio-Plex multiplex array system and human cytokine 27-plex panel. Bars represent mean ± standard deviation of values obtained from two different siRNA target sequences per gene.
Active Caspase-1 Is a Regulator of Unconventional Protein Secretion of IL-1beta confirmed with Multiplex Cytokine Profiling
Multiplex Cytokine Applications

- **Cytokine Expression Profiling**
  - Immunological Profiling of Mononucleocytes in Response to Mitogenic Stimulation
  - Active caspase-1 is a regulator of unconventional protein secretion
  - Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge
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- **Biomarker Validation & Monitoring**
  - Towards Discovery-driven Translational Research in Breast Cancer
  - Multiplex analysis of serum biomarkers in ovarian cancer patients
Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge

Salomon Amar*, Qingde Zhou*, Yazdani Shaik-Dasthaagirisaheb*, and Susan Leeman†

*Department of Periodontology and Oral Biology, School of Dental Medicine, and †Department of Pharmacology, School of Medicine, Boston University, Boston, MA 02118

Contributed by Susan Leeman, November 5, 2007 (sent for review August 21, 2007)
Diet-induced Obesity show low immune response

• Obesity has been suggested to be associated with an increased susceptibility to bacterial infection, only few studies have examined the effect of obesity on the immune response to bacterial infection.

• Mice with diet induced obesity (DIO) and lean control C57BL/6 mice were infected orally or systemically with *P. gingivalis*, and periodontal pathology and systemic immune responses were examined postinfection.

• Peritoneal macrophages harvested from mice with DIO and exposed to *P. gingivalis* exhibited reduced levels of proinflammatory cytokines compared with lean mice and when exposed to *P. gingivalis* LPS treatment.
Global cytokine profile in response to *P. gingivalis* stimulation *in vitro*
In Conclusion

• Diet induced obesity mice infected P. gingivalis exhibited reduced levels of pro-inflammatory cytokines compared with lean mice and when exposed to P. gingivalis LPS treatment

• Together with the findings of significantly reduced recruitment of NF-B to both TNF-alpha and IL-10 promoters 30 min after LPS exposure of macrophages

• Indicated that obesity interferes with the ability of the immune system to appropriately respond to P. gingivalis infection and suggest that this immune dys-regulation participates in the increased alveolar bone loss after bacterial infection observed in mice with diet induced obesity
Studies continued analyzing the intracellular signaling pathways.

Signaling mechanisms involved in altered function of macrophages from diet-induced obese mice affect immune responses

Qingde Zhou\textsuperscript{a}, Susan E. Leeman\textsuperscript{b,1}, and Salomon Amar\textsuperscript{b,1}

\textsuperscript{a}Department of Periodontology and Oral Biology, School of Dental Medicine; and \textsuperscript{b}Department of Pharmacology, School of Medicine, Boston University, Boston, MA 02118

Contributed by Susan E. Leeman, April 29, 2009 (sent for review April 3, 2009)
Lipopolysaccharide-induced increases in cytokines in discrete mouse brain regions are detectable using Luminex xMAP® technology

Subhash C. Datta\textsuperscript{a}, Mark R. Opp\textsuperscript{a,b,c,}\textsuperscript{*}

\textsuperscript{a} Department of Anesthesiology, University of Michigan, Ann Arbor, MI 48109, United States
\textsuperscript{b} Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109, United States
\textsuperscript{c} Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI 48109, United States
• Research focus on the role of cytokines in brain as mediators/regulators of complex physiological processes and behaviors
Bacterial lipopolysaccharide (LPS) was intraperitoneally injected in C57BL/6J mice.

4 h later interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF) was analyzed.

Cytokine level were found to be 4- to 870-fold increased in serum or spleen and increased 1.5- to 16-fold in discrete brain regions.
Protein were extracted in lysis buffer with protease inhibitors by tissue disruption.
Conclusions of the assessment of cytokine protein in discrete mouse brain regions after LPS treatment

• these results demonstrate that 4 h after LPS administration, concentrations of IL-1, IL-6 and TNF increase dramatically in mouse hypothalamus, hippocampus, and brain stem.

• these brain regions are involved in the regulation of multiple complex behaviors and physiological processes, including among others, activation of the hypothalamic-pituitary-adrenal axis and autonomic nervous system activity.

• Cytokines can efficiently measure in tissue homogenates and more cost-effective than individual ELISA assays.
Sample Types – Influence the Assay Performance

- Serum
- Plasma
- Urine
- Saliva
- Tissues
- Lavages
- Amniotic Fluid
- Spermatic Fluid
- Nipple Aspiration Fluid
- Tear Fluid
- Urinary Stones
- Flower Leaves
Bio-Plex Challenges

- Long read times
- High %CV values
- Poor recoveries
- Inconsistent inter-assay concentration values
- Missing beads
- Poor sensitivity

Instrument Obstacles

Assay Performance

Sample Results

IgA Human Serum and Plasma Validation using the Human 7-Plex Isotyping Assays
The Bio-Plex instrument can be ruled out as a source of problems with the Validation Kit.

- Routine monthly validation is recommended

Optics validation

Carryover/fluidics validation

Reporter validation

Classification validation
### Assay Performance Parameters

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<td>86</td>
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**Sensitivity**  
**Precision**  
**Accuracy**  

**Conc in Range**

---

Life Science Group
What to do with difficult to filtrate samples in complex matrices?
What is a Magnetic Assay?

- Bioassay with paramagnetic material

- Super paramagnetic bead are magnetic only in a magnetic field.

- Easier to handle the assays, for washing, compared to filtration or centrifugation
Bead dying is accomplished by the infusion of hydrophobic dyes in organic solvents.
6.5 µm Magnetic Beads

**Core**

- 3.5% Fe$_3$O$_4$

**Coating Layer**

- COOH

Same chemicals used in magnetic bead conjugation to bio-molecules
Defining Cytokine Profiles During Virus Infection

- Centers for Disease Control & Prevention - Influenza Branch

- Define cytokine profile of highly pathogenic avian H5N1 influenza infection

- Cytokine Storm in patients increases the severity of H5N1 infection

- Define cytokine profile in lungs of virally infected mice

- Use cytokine and cytokine receptor knock-out mice to establish which cytokines are important in the control or pathogenicity of H5N1
Optimized procedure for running difficult tissues

- Tissue homogenates were freeze/thawed
- Serum diluent gave the least background, with best recovery
- Due to select agent/BSL3 issues, samples were fixed at the end of procedure
Magnetic beads with difficult to filtrate samples

D6plC  WNV
(Brains halved)

Well Clarified

Not Complete Clarified

Polystyrene

Magnetic

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Magnetic Bead Versatility

• Magnetic beads CAN be used on a vacuum manifold

OR
Detection from complex matrices using magnetic beads

**Bio-Plex COOH beads**

- automated washing steps
- filtration unit
- complex matrices difficult

**Pro**

**magnetic beads**

- complex matrices
- few additional equipment

**Con**

- manual washing steps
Magnetic Bioassays for Better Assays Performance

- Allow separation in various sample matrices
- Remove non-assay related particles
- Use a close incubation environment
- Prepare for semi and full-assay automation
Paramagnetic procedure

Sample homogenate

Add beads

Bead complex I
Paramagnetic procedure

Capture beads
Wash beads
Add reporter antibody
Bead complex

Paramagnetic procedure
Benefit Using Magnetic Beads

- Improve Assay Performance
  - Automate assay wash steps
  - Better dynamic range for Cytokine assays
  - Improve precision (CV%)
- Easy for Protein Conjugation to Beads
  (convenient for customer assay development)
- Automatic Magnetic Bead Washer

Magnetic carrier
Vacuum carrier
Carboxylic acid (COOH)
  • Covalent coupling partner for antigen (or other) amines
  • Source of negative charge for electrostatic adsorption and metal coordination

Autoimmune panels
Vasculitis panel
EBV panel
Syphilis IgG

For more details visit . . . www.bio-rad.com
Magnetic Bead Assay Applications

- Diagnostic Indicators of Immunodeficiency Disorders
- Drug Development
- Vaccine Development
- Autoimmune Disease and Allergy Research
- Infectious Disease Outbreak Surveillance
- Biomarker Validation in Cancer Research
Multiplex Protein Applications

- Cytokine Expression Profiling
  - **BIO-RAD** Immunological Profiling of Mononucleocytes in Response to Mitogenic Stimulation
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  - **CDC** Define cytokine profile of highly pathogenic avian H5N1 influenza infection

- Biomarker Validation & Monitoring
  - **FEBS** Towards Discovery-driven Translational Research in Breast Cancer
  - **BIO-RAD** Multiplex analysis of serum biomarkers in ovarian cancer patients
Bio-Plex Cytokine Assays Are Going Magnetic!

Introducing the Next Generation of Bio-Plex Pro™ Human and Mouse Cytokine Assays

Simplified Workflow
Automate your assays with magnetic separation or simply use the Bio-Plex Pro™ Washer to improve your assay workflow.

All-in-One Kit Convenience
All the necessary reagents required to run the Bio-Plex Pro Cytokine Assay are combined into one convenient kit.

Faster Delivery Option
Through the new Bio-Plex Express assay service, your customized selection of Bio-Plex Pro cytokine assays will ship as early as the next day.

Improved Assay Performance
These new assays have been improved while still offering the same antibodies and buffers as the current non-magnetic Bio-Plex Cytokine Assays.

For more information, visit us at www.bio-rad.com/bio-plex/magnetic.
**Total: 91 Cytokine, Chemokine and Growth Factors Assays**

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<td>VEGF</td>
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</table>

**50 Human Assays**

**33 Mouse Assays**

**Available as x-plex Assays and Express**
Biomarker Validation

Cytokine Profiling - Angiogenesis
( Acute Phase Proteins and Diabetes )
Towards Discovery-driven Translational Research in Breast Cancer

Julio E. Celis, et. al, The Danish Centre for Translational Breast Cancer Research and Institute of Cancer Biology

FEBS Journal, 2005
Antibody Array Data are validated with Bio-Plex Assays

**Antibody Microarray**

Array VI
- N46
- MCP-1α
- IL-1β
- IL-6

Array VII
- T46
- MCP-1α
- IL-1β
- IL-6

**Bio-Plex**

- IL-8
- MCP-1α
- IL-1β

Celis et al. (2005) FEBS Letters
Elevated IL-6 levels were independently confirmed in both experiments.

Additionally, elevated levels of IL-8, IL-1β and MCP-1α were also detected in both experiments.

The antibody arrays required 0.5 ml of fluid for incubation whereas the data from the Bio-Plex can be obtained with only 13-50 μl/well, in one plate.
Are serum cytokines early predictors for the outcome of burn patients with inhalation injuries who do not survive?

Gerd G Gauglitz1,2*, Celeste C Finnerty1,2*, David N Hemdon1,2, Ronald P Mlcak1 and Marc G Jeschke1,2

1Shriners Hospitals for Children, 815 Market Street, Galveston, Texas, 77550, USA
2Department of Surgery, University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas, 77550, USA
* Contributed equally

Corresponding author: Marc G Jeschke, majeschk@utmb.edu

Received: 7 Mar 2008  Revisions requested: 14 Apr 2008  Revisions received: 25 Apr 2008  Accepted: 18 Jun 2008  Published: 18 Jun 2008

Discovery-driven Translational Research for predictors molecular in burn patients

**Cytokine measurements:** Bio-Plex Human Cytokine 17-Plex panel in combination with the **Bio-Plex Suspension Array System**

**PaO2/FiO2 ratio**
The PaO2/FiO2 ratio was used to quantify the degree of abnormalities in pulmonary gas exchange.

**17-plex cytokine assay kit**
IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, G-CSF, CM-CSF, IFN-γ, MCP-1, MIP-1β, and TNF
(a) IL-4 and (d) IL-10 serum levels were significantly increased in the nonsurvivor group at admission compared with survivors (normal IL-4: 0 ± 0 pg/ml, normal IL-10: 1.4 ± 0.3 pg/ml).

(b) Nonsurvivors exhibited a significant increase in IL-6 serum levels 5 to 7 days after admission compared with the survivor group (normal IL-6: 8.7 ± 5 pg/ml).

(c) Nonsurvivors exhibited a significant decrease in IL-7 serum levels 5 to 7 days after admission compared with the survivor group (normal IL-7: 3.8 ± 0.63 pg/ml).

(e) Nonsurvivors exhibited a significant increase in IL-13 serum levels upon hospital admission when compared with the survivor group (normal IL-13: 0.9 ± 0.2 pg/ml).

**Conclusion**

Determination of serum **IL-6, and IL-10** levels upon admission is convenient and simple, and may serve as an early indicator for identifying patients who have a greater risk for mortality after a burn with concomitant inhalation injury.
### Available Magnetic Bead Assays for different Diseases

#### Disease Research Panels

<table>
<thead>
<tr>
<th>Human Cytokines</th>
<th>Human Isotyping</th>
<th>Diabetes</th>
<th>Mouse</th>
<th>Human Acute Phase</th>
<th>Human Angiogenesis</th>
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</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>IgG₁</td>
<td>Adiponectin</td>
<td>Adiponectin</td>
<td>α-2-macroglobulin</td>
<td>Angiopoietin-2</td>
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<tr>
<td>IL-2</td>
<td>IgG₂</td>
<td>Adiponectin</td>
<td>—</td>
<td>CRP</td>
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<td>Ferritin</td>
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<td>IgG₂</td>
<td>C-peptide</td>
<td>Ghrelin</td>
<td>Fibrinogen</td>
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<td>Ghrelin</td>
<td>GIP</td>
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<td>GLP-1</td>
<td>Procalcitonin</td>
<td>Leptin</td>
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<tr>
<td>IL-12 (p70)</td>
<td>IgE</td>
<td>Glucagon</td>
<td>Glucagon IL-6</td>
<td>SAA</td>
<td>PDGF-BB</td>
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<td>IL-6</td>
<td>Insulin</td>
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<td>IFN-γ</td>
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<td>Leptin</td>
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<td>VEGF</td>
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<td>Visfatin</td>
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**Life Science Group**

**BIO-RAD**
**Marker Profiles**

Of the 37 serum proteins studied, serum concentration of 8 markers (IL-6, IL-10, procalcitonin, ferritin, haptoglobin, CRP, angiopoietin-2, and G-CSF) showed significant elevation in the disease group as compared to the healthy controls ($P < 0.003$ to $P < 0.03$). In contrast, 4 serum markers (leptin, PAI-1, PECAM-1, and visfatin) showed significant decrease in the diseased group ($P < 0.01$ to $P < 0.04$). The levels of fibrinogen, SAA, GLP-1, and follistatin showed marked (though not statistically significant) increase in the disease group. The serum profiles of these markers are shown in Figure 3. Similar findings on IL-6 and IL-10 were reported by Yurkovetsky et al. 2007 and Lambeck et al. 2007. The findings on leptin and PAI-1 were also reported by Mor et al. 2005 and Ho et al. 1999, respectively.
Circulating levels of 16 serum markers in ovarian cancer patients and healthy controls
Validation of the Magnetic Beads Assays

Multiplex analysis of serum biomarkers in ovarian cancer patients using Bio-Plex® suspension array.

V. Gupta, C. Reyes, W. Gong, J. Fedynysyn and W. Tan,
Life Science Group, Bio-Rad Laboratories, Hercules, CA, USA.

Abstract:
Ovarian cancer is the fifth leading cause of cancer death among North American women. Since its discovery is often at an advanced stage, early detection is key to successful outcomes. Magnetic bead technology has been adopted for capturing specific antigens/antibodies to detect early stages of ovarian cancer. This study describes validation of detection cancer markers that have been linked to both positive and negative results. The sensitivity and specificity of these markers were compared with the clinical outcomes of patients.

Assay Specifications:
The 27 serum proteins were analyzed in 5 technical replicates (Table 2). The assay, validation and verification of the beads, beads, analytes, pellets, and pellets, were performed in accordance with the manufacturer's instructions. All samples were tested at different concentrations ranging from 20 to 200 ng/ml. The results were normalized to a standard curve and reported as a percentage of the maximum absorbance.

Results:
The results are summarized in Table 3. The precision of the assay was determined by measuring the intraday and interday coefficients of variation (CV). The CV for all assays was less than 10%. The specificity of the assay was determined by analyzing the samples from healthy and cancer patients. The assay was able to detect the presence of the target protein in all samples.

Conclusions:
The Bio-Plex assay is a sensitive and specific tool for the detection of ovarian cancer biomarkers. The assay is simple, rapid, and cost-effective. The results show that the Bio-Plex assay can be used for the early detection of ovarian cancer.
## Available Magnetic Bead Assays for different Diseases

### Disease Research Panels

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<td>IgG₁, IgG₂, IgG₃</td>
<td>Adiponectin, Adipsin, C-peptide, Ghrelin, GIP, GLP-1, Glucagon, IL-6, Insulin, Leptin, PAI-1, Resistin, TNF-α, Visfatin</td>
<td>Adiponectin, Ghrelin, GIP, Glucagon, IL-6, Insulin, Leptin, PAI-1, Resistin, TNF-α</td>
<td>α-2-macroglubulin, CRP, Ferritin, Fibrinogen, Haptoglobin, Procalcitonin, SAA, SAP, Tissue plasminogen activator</td>
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<td>IL-2</td>
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<td>IFN-γ</td>
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Life Science Group
Bio-Plex Diabetes Assay
Obese White Adipose Tissue

Adipsin

- Reduced Levels
- Increased Levels

Visfatin enhances insulin activity

Ghrelin released by the stomach increases food intake and body weight.

C-peptide cleaved from proinsulin to generate insulin

Insulin released by beta cells of islets of Langerhans

Liver releases glucose into blood

Low Blood Glucose

Glucagon released by Alpha cells of islet of Langerhans

High Blood Glucose

Autoimmune attack on pancreas causes T1D

Pancreas

Obese White Adipose Tissue

C-peptide cleaved from proinsulin to generate insulin

Visfatin enhances insulin activity

Ghrelin released by the stomach increases food intake and body weight.

C-peptide cleaved from proinsulin to generate insulin

Visfatin enhances insulin activity

Increased Levels

- TNFα, IL-6, Leptin Resistance, PAI-1

Adipsin

- Vascular and Metabolic Dysfunction

Obese White Adipose Tissue

- Inflammation
- Insulin resistance
- Leptin resistance

Reduced Levels

Adiponectin

Ghrelin released by the stomach increases food intake and body weight.

GLP-1 stimulates insulin secretion and suppresses glucagon secretion

GIP-1 from the gastrointestinal tract stimulates insulin secretion

Hormones of pregnancy can cause Gestational Diabetes

Life Science Group
Pilot study on Diabetes vs. normal serum samples (n=10).

- Patients not controlled for last meal
- Still, 8/12 markers showed significant differences in levels between the groups.
(Pro) Assays – on paramagnetic beads – for Diabetes & Angiogenesis, Acute Phase Protein Research and Cytokine Monitoring
**Online Infos - “Bio-Plex Related”**


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**Multiplex Suspension Array System**

**Phosphoprotein Detection**

**Bio-Plex x-Plex Assays**

**Assay Group and Package Size**

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**Life Science Group**
Multiplex Suspension Array System

A world of information...

Assays

Tools

Software

Support

Technology

BIO-RAD

Literature, Manuals, and Glossary

Bio-Plex Phosphoprotein Detection

New Literature (PDF)
Publications using Bio-Plex cytokine assays

View Archive of Recent Live Webcast Case Studies in Multiplex Protein Analysis

x-Plex Assays

Cytokine Assays
New assays available

Events
Thank you for

Feel free to contact me directly

Christian.Zimmermann@bio-rad.com
More Applications
Capture Molecule Assays

- Sandwich Immuno Assays
- Antigen Binding Assays
- Protein-Interaction Assays
- Ligand-Binding Assays
- Enzym-Substrate Assays
- Protein-DNA Interaction Assays
- Nucleic Acid Hybridisation Assays
The Search for Tumor Biomarkers
Auto-antibody mediated identification of antigens (Gires et al 2004)

Figure 1. Flow chart of the autologous AMIDA screening technology.
The Search for Tumor Biomarkers
Auto-antibody mediated identification of antigens (Gires et al 2004)

- Anti-human IgG with fluorescent label
- Serum antibodies from patient
- Protein identified by AMIDA
- Bead

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